50th ANNIVERSARY OF THE FOUNDING OF
THE AMERICAN SOCIETY OF TROPICAL MEDICINE

VOLUME 2 November 1953 NUMBER 6

THE AMERICAN JOURNAL OF

Tropical Medicine and Hygiene

Combining The American Journal of Tropical Medicine and The Journal of the National Malaria Society

OFFICIAL ORGAN OF
THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

PUBLISHED BIMONTHLY BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE 2, MARYLAND, U.S.A

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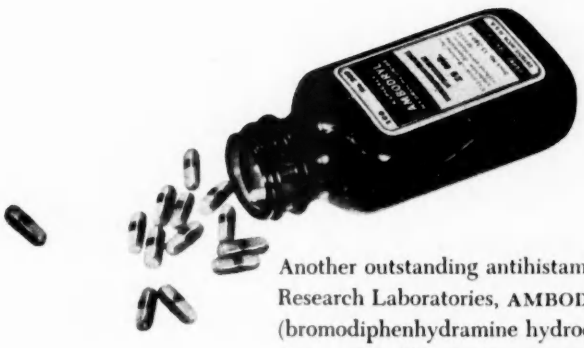

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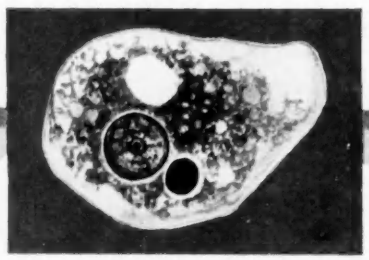

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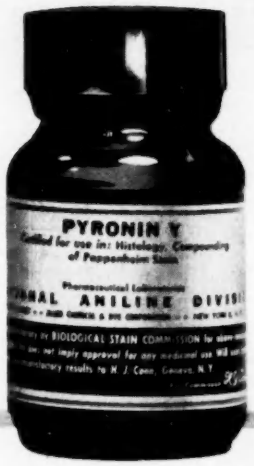

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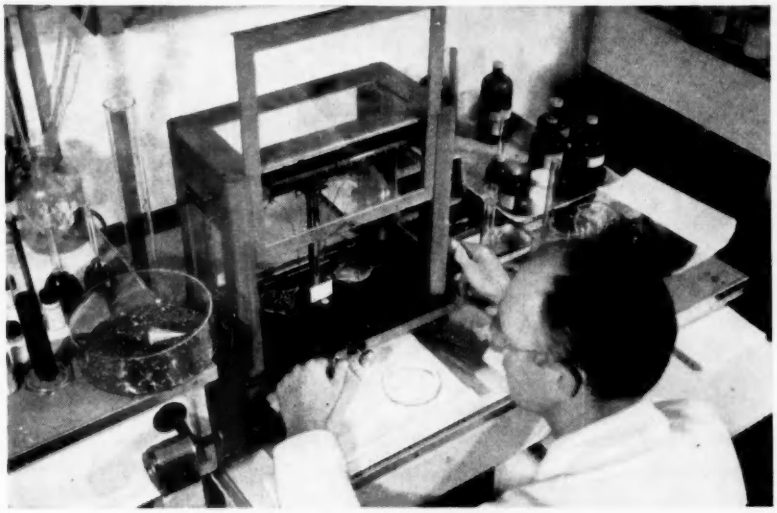

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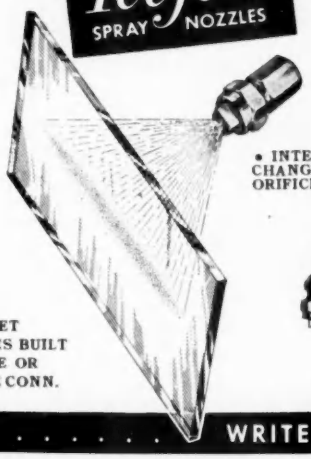

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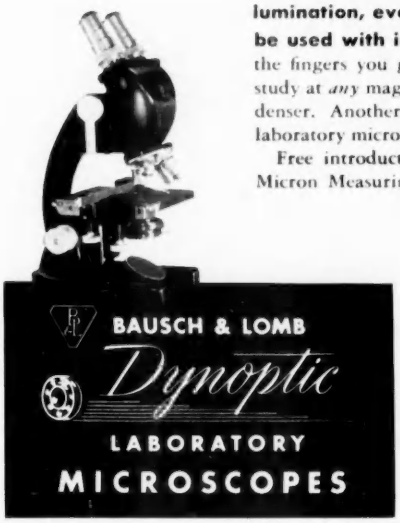

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STUDIES IN HUMAN MALARIA

XXXI. Comparison of Primaquine, Isopentaquine, SN-3883, and Pamaquine as Curative Agents Against Chesson Strain *Vivax* Malaria[1]

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Primaquine, 8-(4-amino-1-methylbutylamino)-6-methoxyquinoline, was first reported by Edgcomb *et al.* (1950) as superior to several closely related 8-aminoquinolines, including pamaquine and isopentaquine, for the cure of Chesson strain *vivax* malaria. Early in 1951, when decisions of policy had to be made as to large-scale procurement of curative antimalarial drugs, it appeared desirable to supplement the above studies with another series in which several drug regimens then under consideration could be directly compared in a large number of subjects concurrently infected and under controlled conditions. This is the report of such a study, carried out in the U. S. Penitentiary, Atlanta, Georgia, in which primaquine, isopentaquine, pamaquine and SN-3883 (Figure 1) were used in treating inmate volunteers infected with the Chesson strain of *Plasmodium vivax* by mosquito bite. Part I deals with trials of selected 14-day regimens of all four drugs; Part II describes limited exploration of shorter and simpler regimens of primaquine and SN-3883.

Part I

METHODS

The methods employed were essentially those described by Coatney *et al.* (1948). The 204 volunteers who took part had no previous history of malaria; all were white males, 21 to 45 years of age, who were aware of the nature of the tests and who received only modest rewards for their participation.

Parasites of the Chesson strain of *P. vivax* were transmitted to the volunteers by the bites of insectary-reared *Anopheles quadrimaculatus* mosquitoes, which were infected approximately 2 weeks earlier by feeding on patients undergoing malarial therapy in a large mental hospital. Each mosquito was allowed to bite three volunteers by a technique of interrupted feeding before being dissected to determine the relative number of sporozoites in its salivary glands. Sporozoite densities were graded on a scale of 0 to 4+. Each volunteer was bitten and rebitten until he had 10 positive bites.

Observations on the volunteers included thick blood smears for parasite esti-

[1] This work was conducted under a contract between the Office of the Surgeon General, Department of the Army and the National Institutes of Health.

[2] Address now—Occupational Health Field Headquarters, 1014 Broadway, Cincinnati 2, Ohio.

mation daily from the eighth day after mosquito bites through the primary attack until at least two successive negative smears had appeared during therapy. Smears were then made every other day until 72 days after bites, twice weekly through day 180, and once weekly through day 270. The first 102 subjects (half of each therapeutic group) were observed clinically for 505 days, the remaining 102 subjects for 350 days, after exposure.

White blood cell counts, including differential counts when totals were below 3,000 or above 12,000 per cu. mm. of blood, were made every other day during therapy and again one week after the last dose of drug. Estimations of hemoglobin, methemoglobin (by modification of the method of Evelyn and Malloy, 1938), and packed red cell volume (hematocrit) were made immediately before drug was started, and again on the seventh and fourteenth day of therapy. Urine samples were examined daily during therapy for changes in color suggestive of intravascular hemolysis.

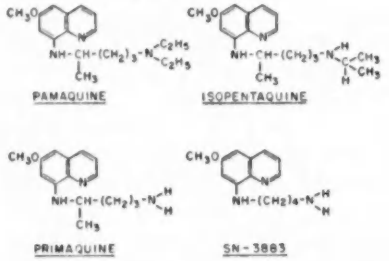

FIG. 1. Structural formulas of the 8-aminoquinoline compounds used in this study

Drugs were given in the following forms: Primaquine (SN-13,272), 8-(4-amino-1-methylbutylamino)-6-methoxyquinoline, was given as the diphosphate salt, which is 56.9 per cent base; isopentaquine (SN-13,274), 8-(4-isopropylamino-1-methylbutylamino)-6-methoxyquinoline, as the monophosphate salt (73.5 per cent base); SN-3883, 8-(4-aminobutylamino)-6-methoxyquinoline, as the hydrochloride (75 per cent base), and pamaquine (SN-971), 8-(4-diethylamino-1-methylbutylamino)-6-methoxyquinoline, as the naphthoate salt, 45 per cent base. All dosages are expressed in terms of base. In each case quinine sulfate was given concurrently.

Treatment of each volunteer was begun on the third day of patent parasitemia, provided that there had been an oral temperature of 101°F. or more. Otherwise treatment was deferred until the fourth or fifth day; treatment was never delayed beyond the fifth day of patent parasitemia even in the absence of fever. All drugs were given (Part I) in four equal doses per day, at 6 a.m., 12 noon, 6 p.m. and 12 midnight for 14 days. The first dose was given at 12 noon and the last at 6 a.m., 14 days later.

Volunteers were hospitalized during acute attacks of malaria until treatment had been completed. They were then allowed to return to their usual prison activity, with no changes in their routines other than those necessitated by

periodic laboratory examinations. First relapses were terminated with the same regimens used during primary attacks; second relapses were terminated with chloroquine, 1.5 gm. (base) over a 3-day period plus primaquine 15 mg. single dose daily for 14 days.

EXPERIMENTS AND RESULTS

The 204 volunteers were infected between 24 April and 27 December 1951. On each bite day the men were divided by lot into six groups, corresponding to the six therapeutic regimens under trial:

Primaquine	20 mg. (base) daily plus quinine 1.0 gm. (base) daily
Primaquine	10 mg. (base) daily plus quinine 1.0 gm. (base) daily
Isopentaquine	60 mg. (base) daily plus quinine 1.0 gm. (base) daily
SN-3883	60 mg. (base) daily plus quinine 1.0 gm. (base) daily
SN-3883	30 mg. (base) daily plus quinine 1.0 gm. (base) daily
Pamaquine	60 mg. (base) daily plus quinine 1.0 gm. (base) daily

Thus when the entire 204 volunteers had been infected and treated, 34 men had been on each of the regimens, under conditions which were comparable in terms of the lots of mosquitoes used as well as season and other environmental factors.

All of the 204 volunteers developed patent parasitemia 9 to 14 days after exposure. Treatment in all patients was begun, as previously indicated, on the third to the fifth day of patency.

Therapeutic results. Erythrocytic parasitemia, fever, and symptoms were promptly relieved by treatment. Relapses occurred to a varying extent in the groups, as summarized in Table 1. It will be noted that there were significantly more relapses with pamaquine than with primaquine, isopentaquine and SN-3883. Thirty-two per cent of the subjects receiving the lowest dose of primaquine and 57 per cent of those receiving the lowest dose of SN-3883 for treatment of first relapse had second relapses; one subject who received pamaquine had a second relapse. None in the other groups relapsed a second time. Only one subject initially given SN-3883 in dosage of 30 mg. had a third relapse.

Intervals from treatment to relapse are shown in graphic form in Figure 2. It will be noted that there was a group of relapses which appeared within the first two weeks after treatment; a second group began to appear about five weeks after treatment.

Toxic reactions. Drug courses were successfully completed in all but three of the 204 subjects. One man, given pamaquine, had such severe nausea and vomiting that treatment was stopped on the ninth day. Another volunteer, who was receiving SN-3883 in dosage of 60 mg. daily, developed iritis and keratitis, with corneal ulceration, which progressed despite topical cortisone and antibiotics. Treatment with SN-3883 was stopped on the ninth day of therapy, although the drug was not conclusively implicated. This man had several subsequent exacerbations and remissions of his eye condition despite the fact that he had no further treatment with SN-3883. In a third patient the drug was discontinued because of proof that the prescribed regimen was not followed.

The principal subjective complaint was abdominal discomfort, including

cramps, abdominal soreness and indigestion. As shown in Table 2, this was most common and most severe in patients given pamaquine, and was notably less frequent in those given primaquine. Other subjective complaints, such as head-

TABLE 1

Incidence of relapses after treatment of early primary attacks of Chesson strain vivax *malaria with various 8-aminoquinoline drugs, given concurrently with quinine sulfate, 1.0 gram (base) per day for 14 days*

DRUG	DAILY DOSE	NO. FIRST RELAPSES/NO. TREATED	PER CENT FIRST RELAPSES	NO. SHOWING SECOND RELAPSES
	mg.			
Primaquine	20	5/34	15	0/5
Primaquine	10	22/34	65	7/22
Isopentaquine	60	12/34	35	0/12
SN-3883	60	3/32*	9	0/3
SN-3883	30	7/34	21	4/7
Pamaquine	60	27/33†	82	0/27

* Two cases excluded because complete course of drug not given.

† One of 34 cases excluded because drug discontinued early.

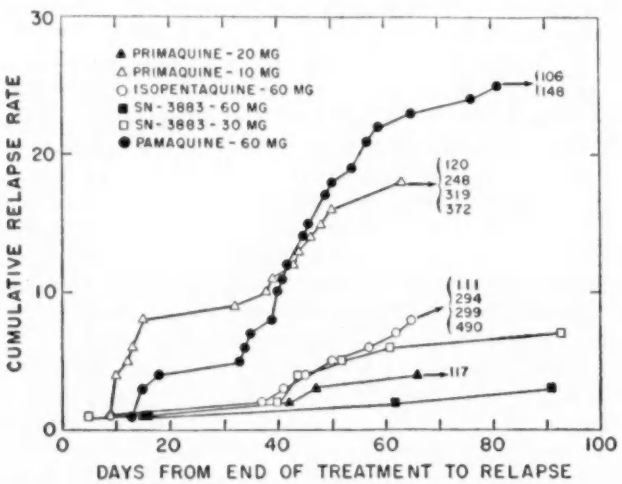

FIG. 2. First relapses following treatment of the initial attacks with certain 8-aminoquinoline compounds, plus quinine, in volunteers infected with the Chesson strain of *vivax* malaria. Figures in brackets indicate days from end of treatment to release where interval was greater than 100 days.

ache and anorexia, could not be correlated with any specific drug regimen. Three subjects complained of transient dizziness. Late in the course of treatment several patients had low-grade fever (below 101°F.) unrelated to malarial parasitemia.

Laboratory studies revealed no evidence of serious disturbances of red or white blood cells. There was no occurrence of intravascular hemolysis. Comparisons of hemoglobin concentrations on the seventh day of therapy with those on the fourteenth day showed that with all drugs except pamaquine the majority of patients treated had an increase in hemoglobin concentration during the last week of therapy, consistent with the recovery period from acute malaria. Detailed comparisons of hemoglobin levels are unwarranted, in view of the varying degrees of malarial parasitemia and hydration during acute attacks of malaria. Data are included in Table 2 to indicate the numbers of patients on each regimen who showed a decrease of hemoglobin between day 7 and day 14 of therapy greater than 1.0 gram per 100 ml. Patients who were given second courses of therapy likewise exhibited no tendency to intravascular hemolysis.

TABLE 2

Toxicity of 8-aminoquinoline compounds given in combination with quinine sulfate 1.0 gm (base) daily for 14 days

DRUG REGIMEN (MG. BASE PER DAY × 14)	NO. SUBJECTS TREATED	ABDOMINAL DISCOMFORT		HEADACHE	CYANOSIS	FEVER	DIZZINESS	HEMOGLOBIN DECREASE >1 GM. PER 100 ML. DAYS 7 TO 14	WHITE BLOOD COUNT DAY 14		WHITE BLOOD COUNT 1 WEEK AFTER TREATMENT	
		Mild	Severe						>12,000	<5,000	>12,000	<5,000
Primaquine, 20 mg.	34	3	1	3	2	1	1	3	6	0	0	5
Primaquine, 10 mg.	34	2	0	4	0	0	0	0	5	0	4	1
Isopentaquine, 60 mg.	34	11	4	2	4	1	1	6	11	1	1	3
SN-3883, 60 mg.	34	6	2	1	10	3	0	6	3	0	0	5
SN-3883, 30 mg.	34	3	3	5	7	2	0	2	3	0	2	6
Pamaquine, 60 mg.	34	4	15	3	9	4	1	7	5	3	0	5

Comparison of packed red blood cell volumes on the seventh and fourteenth day of therapy was consistent with the hemoglobin values and indicated no evidence of serious red blood cell destruction.

All of the compounds tested caused varying degrees of methemoglobinemia. Values in terms of per cent of total hemoglobin were compared on the last day of therapy. There were wide variations from patient to patient within each therapeutic group, but the average methemoglobin concentration was highest in subjects given pamaquine, and lowest in those given primaquine. Clinically apparent cyanosis was noted in 9 of 34 men given pamaquine, 10 of 34 given SN-3883 (60 mg.), and in 2 of 34 subjects given primaquine (20 mg.). No correlation was noted between the degree of methemoglobinemia or cyanosis and the occurrence of headache or abdominal discomfort.

White blood cell counts in most patients were normal or low at the height of the acute malarial attack. By the last day of treatment a considerable proportion of the patients, representing all drug regimens, had moderate leukocytosis (Table 2). Counts returned to normal, being normal in nearly all patients one week after treatment.

DISCUSSION

Chesson strain *vivax* malaria infections of the severity initiated in these studies can be expected to produce a series of closely spaced relapses when noncurative drugs are used (Coatney *et al.*, 1950). In Part I of our tests, primaquine and SN-3883 appeared definitely superior to pamaquine and isopentaquine in their ability to cure Chesson strain *vivax* malaria when given in combination with quinine sulfate. In general, our results corroborate those of Edgcomb *et al.* (1950) for primaquine, of Alving (1948) for isopentaquine, and unpublished results of Alving (1952) for SN-3883.

Although SN-3883, in the dosages used, was as effective as primaquine in terms of ability to cure, the higher incidence of annoying side-actions with SN-3883, as contrasted with almost complete tolerance of patients to primaquine, makes the latter drug preferable for routine curative therapy. The low toxicity of primaquine in doses below 30 mg. as pointed out by Clayman *et al.* (1952) was corroborated by our studies.

The 8-aminoquinoline drugs are not efficient agents in destroying the erythrocytic parasites of *vivax* malaria and there is a suggestion in our experiment that even the combination of 1.0 gram (base) of quinine per day for 14 days did not eradicate all erythrocytic parasites in all patients. The evidence for this lies in the occurrence of a small group of relapses 5 to 18 days after therapy distinct from the major group of relapses beginning about 5 weeks after therapy (Figure 2). This suggests two relapse mechanisms. The relapses which occurred early are probably recrudescences caused by descendants of erythrocytic parasites which survived exposure to quinine. Edgcomb (1950) describes what is apparently the same response when primaquine is used alone. The second and larger group represents true relapses. While the number of recrudescences in our series does not invalidate the results of the test, it does provide a strong argument for using a potent agent such as chloroquine against erythrocytic parasites in combined therapy regimens (cf. Garrison *et al.*, 1952).

Part II

About the time our tests showed primaquine and SN-3883 to be superior to pamaquine or isopentaquine for the cure of Chesson strain *vivax* malaria, studies being carried on in Army installations at Fort Benning, Georgia, and Fort Knox, Kentucky, gave results which showed that primaquine in single daily doses of 15 mg. for 14 days given in combination with chloroquine (1.5 gm. base in 3 days) was curative against Korean *vivax* malaria (Garrison *et al.*, 1952). The question then arose as to whether cure of Chesson *vivax* might be obtained if the treatment time were cut in half, that is from 14 to 7 days, and the drug given in single daily doses. Studies to determine this were accordingly arranged.

METHODS

The methods of infecting, treating, and observing volunteers were the same as outlined in Part I, except for details of drug administration.

EXPERIMENT AND RESULTS

Thirty volunteers were infected; the first group on 18 August and the second on 20 August 1952. The men bitten on each bite day were divided by lot into three groups, corresponding to the three therapeutic regimens under trial:

Primaquine 30 mg. (base) single dose daily × 7
Primaquine 20 mg. (base) single dose daily × 7
SN-3883 60 mg. (base) single dose daily × 7

In addition each patient received 1.5 gm. (base) of chloroquine during the first 3 days of treatment (0.6 gm. initial dose followed in 6 hours by 0.3 gm., then 0.3 gm. once daily for 2 days) for the management of the acute attack.

All 30 volunteers developed patent parasitemia 10 to 14 days after exposure; 10 men were treated with each regimen, beginning on the third to the fifth day of patent parasitemia.

TABLE 3

Incidence of relapses after treatment of early primary attacks of Chesson strain vivax *malaria with primaquine and SN-3883, each given in a single dose daily for 7 days; chloroquine was given concomitantly during the first 3 days*

DRUG	DAILY DOSE	NO. FIRST RELAPSES/NO. TREATED	PER CENT FIRST RELAPSES
	mg.		
Primaquine	30	9/10	90
Primaquine	20	8/10	80
SN-3883	60	10/10	100

Therapeutic results. Erythrocytic parasitemia, fever and symptoms were promptly relieved by treatment. A high percentage of relapses occurred in all three groups (Table 3); these appeared around 40 to 50 days after the last dose of chloroquine, which is about the time when chloroquine blood levels, following this dosage, fall below the suppressive level.

Toxic reactions. There were no untoward toxic reactions.

DISCUSSION

These results indicate that experimental Chesson strain infections of the intensity induced in these volunteers by the bites of 10 heavily infected mosquitoes are not cured by regimens which have proved curative against naturally-acquired Korean *vivax* infections (Garrison *et al.*, 1952). From the results one cannot determine whether this reflects differences in strain or in intensity of exposure.

GENERAL SUMMARY

In Part I of this study six therapeutic regimens employing four different 8-aminoquinoline derivatives were tested under strictly controlled conditions in mosquito-transmitted Chesson strain *vivax* malaria, experimentally induced in volunteers in a Federal penal institution. Thirty-four men were treated with

each regimen. Test drugs were given every 6 hours for 14 days, in combination with quinine sulfate, 250 mg. of base every 6 hours for the same period. The relapse incidence after primary attacks was as follows:

Primaquine	20 mg. (base) daily	15 per cent
Primaquine	10 mg. (base) daily	65 per cent
Isopentaquine	60 mg. (base) daily	35 per cent
SN-3883	60 mg. (base) daily	9 per cent
SN-3883	30 mg. (base) daily	21 per cent
Pamaquine	60 mg. (base) daily	82 per cent

Although toxic manifestations were not alarming with any of the compounds, primaquine at the effective dosage of 20 mg. per day was exceptionally well tolerated, and under the conditions of the test was regarded as the best of the four drugs.

Part II of the study dealt with the trial of three therapeutic regimens employing two different 8-aminoquinolines under the same conditions as outlined above. Ten men were treated with each regimen. The test drugs were given in a single dose daily for 7 days; chloroquine, 1.5 gm. (base) total dose, was given concomitantly during the first 3 days for the management of the acute attack. Relapse incidence after the acute attack was as follows:

Primaquine	30 mg. (base) single dose daily	90 per cent
Primaquine	20 mg. (base) single dose daily	80 per cent
SN-3883	60 mg. (base) single dose daily	100 per cent

There were no toxic manifestations, but the high relapse incidence indicates the limited curative efficacy of these shorter regimens against severe Chesson strain *vivax* infections and consequently that they are not generally applicable curative regimens.

Acknowledgments

The authors desire to make special acknowledgments and extend thanks to Mr. James V. Bennett, Director of the Federal Bureau of Prisons, to Mr. William H. Hiatt, Warden of the U. S. Penitentiary, Atlanta, Georgia, to Dr. H. M. Janney, Chief Medical Officer of that institution, and to their respective staffs who made these studies possible.

Thanks are also extended to the Medical Service of the Army for providing some of the laboratory and nursing personnel who contributed so much to the operation of the overall project.

We cannot overemphasize the contribution made by the inmate volunteers who willingly bore the discomforts and uncertainties of malaria and experimental drugs. We extend our thanks to them for their contribution to this study.

REFERENCES

ALVING, A. S., 1948. Pentaquine (SN-13,276) and isopentaquine (SN-13,274), therapeutic agents effective in reducing relapse rate in *vivax* malaria. Proceedings of the Fourth International Congresses on Tropical Medicine and Malaria, Washington, D. C., May 10–18, pp. 734–741.

ALVING, A. S., 1952. Personal communication.

CLAYMAN, C. B., ARNOLD, J., HOCKWALD, R. S., YOUNT, E. H., JR., EDGCOMB, J. H. AND

ALVING, A. S., 1952. Status of primaquine. 3. Toxicity of primaquine in Caucasians, *J.A.M.A.* **149:** 1563–1568.

COATNEY, G. R., COOPER, W. C. AND RUHE, D. S., 1948. Studies in human malaria. VI. The organization of a program for testing potential antimalarial drugs in prisoner volunteers, *Am. J. Hyg.* **47,** 113–119.

COATNEY, G. R., COOPER, W. C. AND YOUNG, M. D., 1950. Studies in human malaria. XXX. A summary of 204 sporozoite-induced infections with the Chesson strain of *Plasmodium vivax*, *J. Nat. Malaria Soc.* **9:** 381–396.

EDGCOMB, J. H., ARNOLD, J., YOUNT, E. H., JR., AVLING, A. S., EICHELBERGER, L., JEFFREY, G. M., EYLES, D. AND YOUNG, M. D., 1950. Primaquine, SN-13272, a new curative agent in *vivax* malaria: a preliminary report, *J. Nat. Malaria Soc.* **9:** 285–292.

EVELYN, K. A. AND MALLOY, H. T., 1938. Microdetermination of oxyhemoglobin, methemoglobin, and sulfhemoglobin in a single sample of blood, *J. Biol. Chem.* **126:** 655–662.

GARRISON, P. L., COKER, W. G., JASTREMSKI, B., COATNEY, G. R., ALVING, A. S. AND JONES, R., JR., 1952. Status of primaquine. 2. Cure of Korean *vivax* malaria with pamaquine and primaquine, *J.A.M.A.* **149:** 1562–1563.

KOREAN *VIVAX* MALARIA

I. Natural History and Response to Chloroquine*

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As a result of the military action in Korea, large numbers of nonimmune individuals returned to this country after exposure to *Plasmodium vivax* in a limited geographical area for a relatively short time. This circumstance provided a unique opportunity to observe the natural history of the disease and to study its response to therapy under conditions in which reinfection could not occur.

Because *vivax* malaria was known to be endemic in Korea, U. S. troops were given chloroquine once weekly beginning with the onset of hostilities and that regimen was continued during the transmission seasons. This medication has largely prevented clinical attacks of malaria in troops in the field. While chloroquine is a highly effective suppressive agent, it does not destroy the tissue stages of the parasite with the result that attacks occur after suppressive medication is discontinued.

In the spring of 1951 many of the troops who had been in the combat zone since the beginning of hostilities were replaced. As they were relieved, men returned to this country, chloroquine suppression was discontinued when they left the theater, and shortly after arrival here acute attacks of malaria began to occur. The number of new cases of malaria admitted to military hospitals in the United States increased progressively to a peak of 629 new cases per week in August, 1951 (Alving *et al.*, 1952).

In the summer of 1951, when it became apparent that malaria was a major problem in Korean veterans, large scale studies designed to determine the optimum therapy of malaria of Korean origin were initiated at Fort Benning, Georgia and Fort Knox, Kentucky by the Research and Development Board of the Office of the Surgeon General of the Army. Additional units were established at Fort Dix, New Jersey; Fort Meade, Maryland; Camp Breckenridge, Kentucky; and the Naval Hospital at Camp Lejeune, North Carolina, a short time later. The studies were designed to compare the efficacy of primaquine, a new 8-aminoquinoline (Edgcomb *et al.*, 1950), with the efficacy of pamaquine (Plasmochin) in reducing the relapse rate of *vivax* malaria of Korean origin, and to determine the optimum dosage regimen for primaquine. In the course of these studies

* These studies were conducted at Fort Benning, Georgia and Fort Knox, Kentucky. This work was supported (in part) by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army.

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[2] Department of Medicine, University of Pennsylvania.

[3] Laboratory of Tropical Diseases, National Microbiological Institute, National Institutes of Health.

[4] Department of Medicine, University of Chicago.

3,169 cases of *vivax* malaria were treated at several military installations. The results are presented in this series of papers. It is the purpose of this paper to describe the natural history of Korean *vivax* malaria as observed at Fort Benning and Fort Knox.

MATERIAL AND METHODS

Between 15 July, 1951, and 1 November, 1952, 1,513 individuals who acquired malaria in Korea were treated at the time of an acute attack. The diagnosis was established in every case by demonstrating parasites in stained smears of the peripheral blood in each of two nonconsecutive smears. All patients were interviewed and examined at the time of admission, the hemoglobin concentration, white blood cell count, and urinalysis were determined. Additional laboratory studies were done when deemed necessary.

When the diagnosis was established and the temperature exceeded 101°F. (oral) patients were placed on a regimen of 1.5 gm. of chloroquine (base) in 3 days. During the first 24 hours 3 doses of 0.3 gm. were administered, followed by a single dose of 0.3 gm. daily for 2 days. In 512 patients, this regimen was the only specific therapy; 1,213 patients were treated with primaquine or other 8-aminoquinolines in addition to chloroquine.

All patients were hospitalized. When treatment was completed, they were discharged to duty and followed as out-patients at approximately 6-week intervals. At the time of each follow-up visit, the patients were interviewed and a thick film of peripheral blood was examined for parasites. The men also reported to the hospital for consultation and blood smears any time they suspected a relapse of malaria. All of the patients included in this report were followed for more than four months after treatment. (A small percentage of the patients was followed by mail after discharge from the Army.) Those patients who relapsed after treatment with chloroquine alone were again treated with chloroquine at the time of each relapse in order that we might observe the natural history of this disease[5].

Three hundred and fifty-five of the 512 patients treated with chloroquine alone were followed for more than four months after treatment. This analysis of the natural history of Korean *vivax* malaria is based, for the most part, on observations of the disease in 355 patients. Certain data on the entire group of 1,513 patients were utilized in the analysis of those portions of the natural history that occurred before treatment with curative agents.

RESULTS

A. *Natural history*

It is well established that *vivax* malaria exhibits two main patterns of clinical activity, depending upon the geographical origin of the parasite (Coatney *et al.*, 1950, 1950a). These two patterns of activity are represented diagrammatically in Figure 1. Strains of *P. vivax* that originate in tropical areas characteristically produce clinical attacks of malaria at frequent intervals throughout the year.

[5] Late in the summer of 1952, as the study was nearing completion, some patients who relapsed after treatment with chloroquine alone were treated with one of the curative drugs.

Strains of *vivax* that originate in temperate zones exhibit a bimodal pattern of clinical activity. Clinical attacks following infections acquired in the summer,

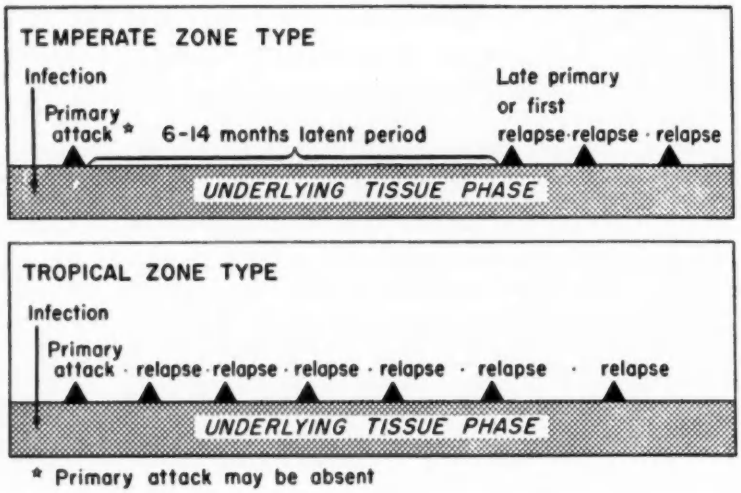

FIG. 1

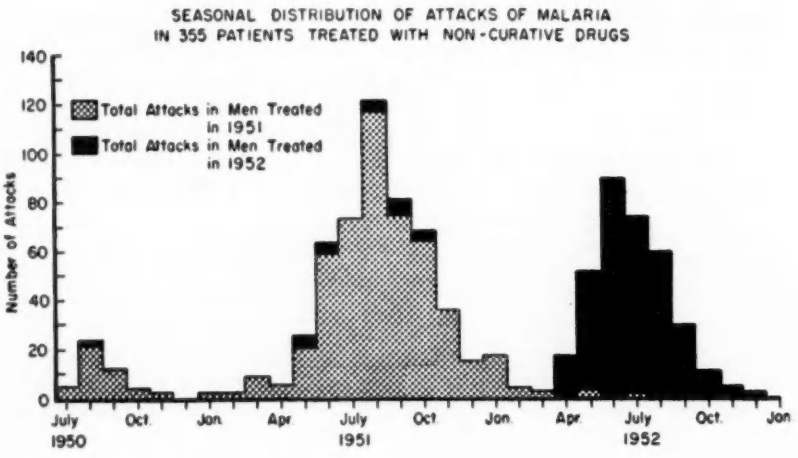

FIG. 2

when mosquitoes are available for transmission, recur during the next summer; the parasite remains latent during the colder seasons of the year.

1. *Pattern of clinical activity.* The total number of attacks of malaria that occurred in 355 patients treated with noncurative drugs only and followed for more

than four months after treatment is shown in Figure 2. Attacks that occurred before these patients entered the study are included when their descriptions of attacks were sufficiently clear.

Two hundred and fifty-eight of these patients developed malaria in this country and were treated between 15 July and 31 December, 1951; and an additional 97 patients were treated between 1 January and 1 August, 1952. All of the patients treated in 1951 were exposed to malaria in Korea in 1950 and more than 80 per cent were followed for more than six months after treatment; most of them were followed for more than one year. These 258 patients experienced a total of 715 attacks of malaria, or an average of 2.8 attacks per patient. In individual patients the number of attacks varied from 1 to 9 (Table 1). The seasonal distribution of clinical attacks in this group of patients is shown in Figure 2. Thirty-six men (14 per cent) gave a convincing description of having an acute attack of malaria in Korea during the summer of 1950[6].

TABLE 1

Number of acute attacks of malaria in 258 patients treated with noncurative drugs and followed more than 4 months

NUMBER OF ATTACKS	NUMBER OF PATIENTS
1	56
2	82
3	37
4	30
5	19
6	14
7	5
8	4
9	2

In the middle of October, 1950, with the advent of cold weather the weekly administration of chloroquine for suppression of malaria was discontinued in Korea; suppressive chloroquine was resumed early in April, 1951, shortly before the start of the malaria transmission season. Despite the fact that chloroquine suppression was discontinued in October, 1950, very few acute attacks of malaria occurred until the spring of 1951. Starting in March, 1951, the number of attacks increased in frequency each month until August, and decreased progressively thereafter. In patients treated in 1951 (stippled area Figure 2) the last attack of malaria occurred in July, 1952.

It is apparent from the data in Figure 2, and similar data reported by Kehoe and Chandler (1952) and by Hale and Halpenny (1953), that Korean *vivax* malaria exhibits a bimodal pattern of clinical activity that is characteristic of strains that originate in temperate regions, rather than the pattern of continuous

[6] When these men were questioned, it was found that almost without exception, they had failed to receive suppressive chloroquine because of the tactical situation, breakdown in chloroquine discipline, or because they deliberately failed to take the drug.

activity throughout the year that characterizes strains that originate in the tropics.

The pattern of clinical activity during 1951, shown in the stippled area Figure 2, differs from the natural pattern as the result of two factors: (1) only a few Korean veterans were in this country during the spring and early summer of 1951, because the return of troops from Korea on a large scale did not begin until May of that year; (2) in many of the men treated in 1951 the early attacks

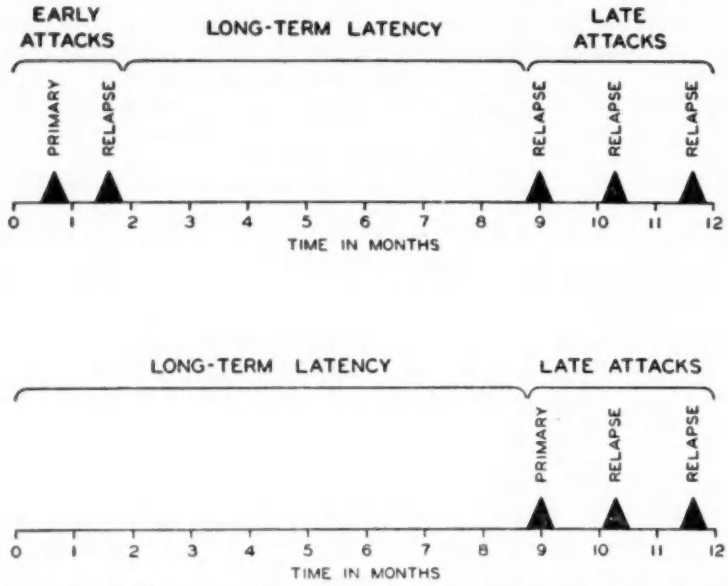

FIG. 3. Upper portion shows long-term latency in temperate zone vivax malaria following treatment of the early attacks. Lower portion shows long-term latency following suppression of the primary attacks (From Coatney, Cooper, Ruhe, Young, and Burgess, 1950).

after the long latent interval were suppressed by chloroquine given in Korea from April, 1951, until they left the theater. Hence it is probable that in the natural pattern of clinical activity more attacks occur during the spring and early summer than is shown in the 1951 data. This interpretation is supported by analysis of the 1952 data (black area Figure 2) which shows that the maximum number of clinical attacks occurred in June. Data presented in the fifth paper of this series confirms this opinion also (Coatney *et al.*, 1953).

Because Figure 2 represents a composite picture of the occurrence of clinical attacks of malaria in men with a varied exposure to infection and variable amounts of suppressive treatment, the natural history of this disease cannot be defined precisely without further analysis. However, by arranging patients into groups with similar exposure to infection and similar suppressive treatment,

and by adding data from patients treated with curative drugs whenever applicable, it is possible to define many of the details of the natural history. The terminology employed is that used by Coatney *et al.* (1950) in their description of the natural history of the disease produced by the St. Elizabeth strain of *Plasmodium vivax* (Figure 3).

(a) *Early attacks.* The duration of the prepatent period cannot be defined because sporozoite inoculation occurred in the field. The primary attack was suppressed by chloroquine in most of the patients so the frequency with which it occurs naturally cannot be determined. Six of the 36 patients who had an early primary attack during the summer of 1950 (see footnote under *Pattern of clinical activity*) attested to a second attack 5 to 8 weeks later. These second attacks may be interpreted as (1) early relapses or (2) recrudescences of persistent parasitemia.

TABLE 2

Length of the latent interval in 88 subjects who experienced an early primary attack of malaria in Korea and received no suppressive medication the following spring before onset of clinical activity

MONTHS BETWEEN PRIMARY ATTACK AND FIRST LATE ATTACK	NUMBER OF PATIENTS	PER CENT OF TOTAL
6	3	3
7	4	5
8	10	11
9	23	26
10	20	23
11	13	15
12	8	9
13	4	5
14	3	3

If the latter interpretation is accepted, and experience favors that interpretation (see Coatney *et al.*, 1950) the second attacks resulted from inadequate treatment of the first attack plus a break-down in chloroquine suppression subsequently.

(b) *Long-term latency.* The duration of long-term latency can be determined by measuring the interval between the primary attack and the first late attacks in patients who did not receive suppressive chloroquine after October of the year in which they were infected. In the entire group of patients studied (including those who were treated with one of the curative drugs later in the course of their disease) there were 88 patients who gave a definite history of having a primary attack and who took no suppressive chloroquine after October of the year in which they were exposed. The interval between the primary attack and the first late attack in these 88 patients is shown in Table 2. The period of long-term latency varied from 6 to 14 months with a mean of 9.8 months. Nearly 50 per cent of the infections were latent for 9 to 10 months.

(c) *Late attacks.* The seasonal distribution of the *first* late attacks after the period of long-term latency is shown in Figure 4. The time of the first late attack could be determined in 486 patients who were exposed to infections in Korea

during a single transmission season (1950 or 1951) and took no suppressive chloroquine after October. In these patients the first late attack occurred as early as January and as late as October. In more than 50 per cent of the patients exposed during a single transmisson season the first late attack occurred in May or June of the following year.

The data presented in the stippled area of Figure 2 suggest that naturally acquired Korean *vivax* malaria terminates spontaneously within two years after sporozoite infection. Critical evaluation of the duration of clinical activity is not

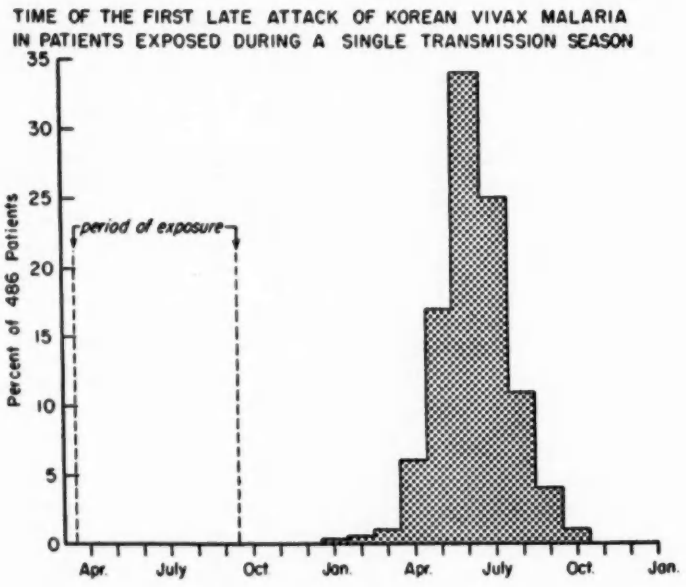

FIG. 4

possible from these data because all of the patients who acquired malaria in 1950 and were treated in 1951 were not followed through the summer of 1952. However, within this group there were 72 men who were exposed to infection *only* during 1950; each had one or more relapses in 1951, and each was followed until September 1952. The time of the *last* clinical attack in each of these 72 men is shown in Figure 5. In 5 patients the last clinical attack occurred in July, 1951, one year after they had entered the endemic area. The last clinical attack in this group occurred in March, 1952, 21 months after the patient had entered the endemic area, and 17 months after the end of the transmission season of that year (1950).

The seasonal distribution of the *first* and the *last* late attacks of malaria is shown as cumulative frequency ogives in Figure 6. The time of the first late attack was known for 489 patients who received no suppressive chloroquine in the

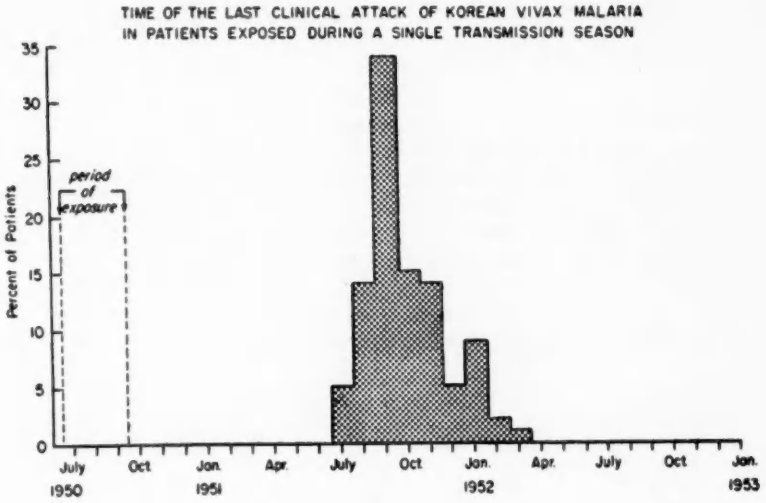

FIG. 5

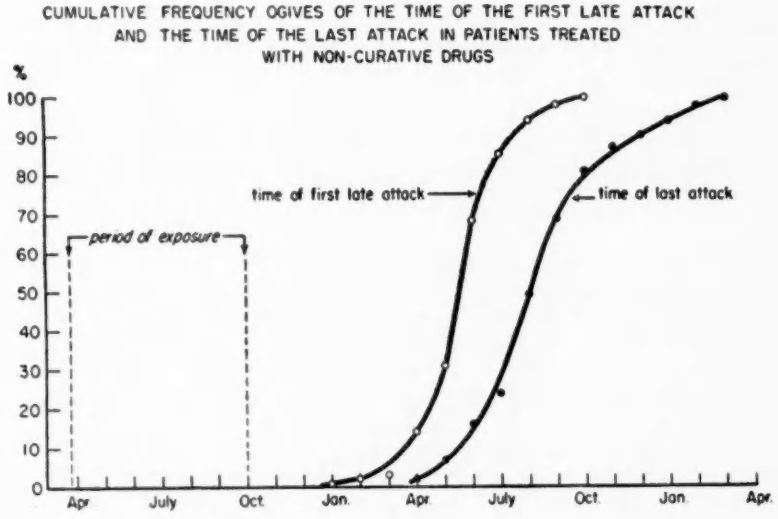

FIG. 6

spring following exposure to infection. The time of the last late attack is based on data from 183 patients. These patients were in Korea during a single transmission season, their acute attacks were treated with noncurative drugs and they were followed for more than four months. The figure shows that in naturally ac-

quired infections late attacks would occur from January to the following March (14 months), 90 per cent of these attacks would occur between April and December (10 months), and 50 per cent between May and October (6 months). The fact that the curves are nearly parallel indicates that late attacks in any one individual would tend to occur over a period of 3 to 5 months.

A second period of long-term latency has not been observed in any individual who was exposed to malaria during a single transmission season. Hence it may be concluded that naturally acquired Korean *vivax* malaria terminates spontaneously within two years after sporozoite inoculation. This probably reflects the duration of the life span of the tissue stages of the parasite (Coatney *et al.*, 1950).

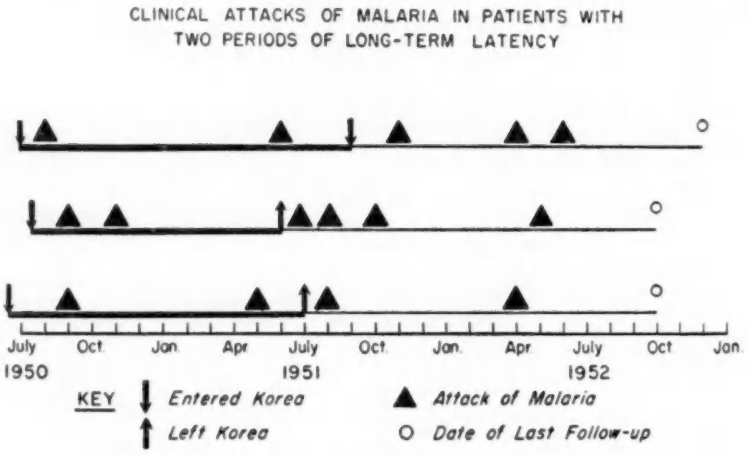

FIG. 7

(d) *Reinfection and superinfection.* In 3 patients who were exposed to malaria in Korea during the summers of 1950 and 1951, there was a second period of long-term latency. The sequence of clinical attacks in these patients is shown in Figure 7. All had primary attacks in Korea in 1950, followed by a period of long-term latency. They developed late relapses in the summer of 1951, which were followed by a second period of extended latency lasting 211 to 247 days. All developed late relapses in the spring of 1952.

Because these patients were in Korea during the transmission seasons of 1950 and 1951, we believe that the second period of long-term latency was the result of a second sporozoite inoculation during the summer of 1951.

B. *Racial immunity*

It is well recognized that the American Negro has a high degree of immunity to many strains of *P. vivax*. In the entire group of 1,513 patients with Korean malaria only five per cent were Negroes (77 patients). This figure is lower than would be expected on the basis of the proportion of Negroes in the troops in

Korea, suggesting that the American Negro has considerable immunity to Korean *vivax* malaria. In those Negroes who developed the disease, the natural history and clinical attacks did not seem to be different from infections in white men, but the group was too small for a detailed analysis.

C. *Clinical picture of the acute attack*

Most of the acute attacks of malaria that were seen by us were late attacks. Many of the patients were seen during their first late attack, and for those who had not experienced an early primary attack this was their first clinical episode

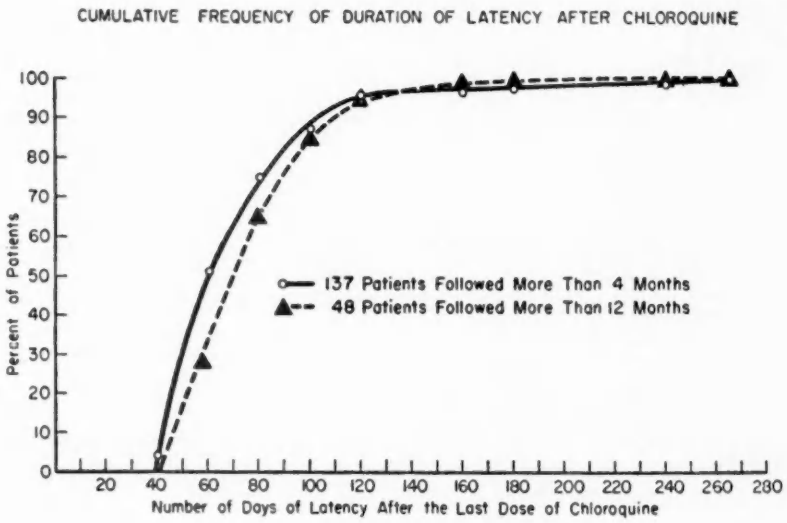

FIG. 8

of malaria. The clinical manifestations of the acute attack, which have been adequately described by Kehoe and Chandler (1952), did not differ significantly from the acute attacks of other strains of *vivax* malaria.

D. *Relapse rate and duration of latency after treatment with chloroquine*

The clinical manifestations of the acute attack were promptly controlled by chloroquine. One hundred and thirty-seven (39 per cent) of the 355 patients treated with chloroquine and followed for more than four months after treatment relapsed at least once, 11 patients relapsed twice, and 2 patients relapsed 3 times. The average duration of the latent interval after treatment (measured from the day after the last dose of drug until the day parasites were first demonstrated) was 68.6 days and the range was 39 to 247 days. The duration of the latent interval after treatment is shown as a cumulative frequency curve in Figure 8. The broken line represents the duration of the latent interval in a group of 48

patients who were followed for more than one year after treatment, and the solid line represents the duration of the latent interval in the entire group of 137 patients who were followed for more than 4 months after treatment. Among the patients followed more than one year, over 90 per cent of those who relapsed did so within 120 days after treatment. The cumulative frequency curve for the larger group, where the follow-up was four months or longer is essentially the same as the curve for the patients who were followed for more than a year. The results indicate that with naturally acquired infections of Korean *vivax* malaria, approximately 95 per cent of relapses after treatment with chloroquine occur within four months. Hence a follow-up period of four months is adequate in studies designed to evaluate the effect of treatment on the relapse rate after treatment of late attacks.

TABLE 3

Comparison of the natural history of Korean and St. Elizabeth strain vivax *malaria*

TYPE OF DATA	KOREAN	ST. ELIZABETH*
Prepatent period	—	11–18 days
Early relapse	Yes	Yes
Duration of long-term latency	5–14 months	5–12 months
Total duration of clinical activity	12–18 months	12–14 months

* Data from Coatney *et al.*, 1950.

SUMMARY AND CONCLUSIONS

Korean *vivax* malaria exhibits a bimodal pattern of clinical activity and a period of long-term latency similar to other strains of *Plasmodium vivax* originating in temperate climates. It was possible to define many aspects of the natural history of this strain with a fair degree of accuracy in large numbers of men infected in the field. The pattern of clinical activity of Korean *vivax* malaria is similar in all major respects to the pattern of the St. Elizabeth strain (Table 3). In the absence of reinfection during a second transmission season, Korean *vivax* malaria acquired in the field terminates spontaneously within two years. Negroes appear to be less susceptible to infection with this strain of *vivax* malaria than Caucasians. A follow-up period of four months is adequate to determine the effect of a therapeutic agent on the relapse rate, when the majority of attacks are late attacks.

REFERENCES

Alving, A. S., Arnold, John, Robinson, D. H., 1952. Status of primaquine. 1. Mass therapy of subclinical vivax malaria with primaquine, *J.A.M.A.* **149:** 1558–1562.

Coatney, G. R., Cooper, W. C., Ruhe, D. S., Young, M. D., Burgess, R. W., 1950. Studies in human malaria. XVIII. The life pattern of sporozoite-induced St. Elizabeth strain vivax malaria, *Am. J. Hyg.* **51:** 200–215.

Coatney, G. R., Cooper, W. C., and Young, M. D., 1950a. Studies in human malaria. XXX. A summary of 204 sporozoite-induced infections with the Chesson strain of *Plasmodium vivax*, *J. Nat. Malaria Soc.* **9:** 381–396.

COATNEY, G. R., ALVING, A. S., JONES, R., JR., HANKEY, D. D., ROBINSON, D. H., GARRISON, P. L., COKER, W. G., DONOVAN, W. M., DI LORENZO, A., MARX, R., SIMMONS, I. H., 1953. Korean *vivax* Malaria. V. Cure of the infection by primaquine administered during long-term latency, *Am. J. Trop. Med. & Hyg.* **2:** 985–988.

EDGCOMB, J. H., ARNOLD, J., YOUNT, E. H., JR., ALVING, A. S., EICHELBERGER, L., JEFFERY, G. M., EYLES, D., AND YOUNG, M. D., 1950. Primaquine, SN 13,272, a new curative agent in vivax malaria: a preliminary report, *J. Nat. Malaria Soc.* **9:** 285–292.

HALE, T. R. AND HALPENNY, G. W., 1953. Malaria in Korean veterans, *Canad. M. A. J.* **68:** 444–448.

KEHOE, E. L., AND CHANDLER, B. F., 1952. Tertian malaria at Walter Reed Hospital, *Mil. Surgeon* **111:** 325–335.

KOREAN *VIVAX* MALARIA

II. Curative Treatment with Pamaquine and Primaquine*

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Edgcomb and others in a preliminary report (1950) estimated that primaquine (SN-13,272), a new curative antimalarial agent, was approximately four times as effective as pamaquine (Plasmochin), a closely related 8-aminoquinoline, in the therapy of heavily seeded, experimental infections of Chesson strain *vivax* malaria, and stated that it was superior to both pentaquine and isopentaquine. These findings have been confirmed and expanded in tests on inmate volunteers at the U. S. Penitentiary in Atlanta, Georgia by Coatney and his group (Cooper *et al.*, 1953) and at the Illinois State Penitentiary, Stateville, Illinois by Alving and his co-workers (1953). The enlarged studies indicated that primaquine is at least four to six times as active as pamaquine when these drugs are compared on an equal weight basis. Although the maximum tolerated dose of primaquine in Caucasian subjects is several-fold that of pamaquine, the drugs have approximately equal toxicity at the lower dosage schedules recommended for therapy (Clayman *et al.*, 1952). Both compounds produce hemolytic anemia in approximately five per cent of Negroes and other deeply pigmented racial groups, when administered daily in dosages of 30 mg. base or more (Hockwald *et al.*, 1952).

In the summer of 1951 therapeutic trials designed to evaluate the effectiveness of primaquine, compared to that of the older drug, pamaquine, for the curative treatment of *vivax* malaria of Korean origin, were initiated at Fort Knox, Kentucky and Fort Benning, Georgia. This paper presents the final evaluation of the results after 18 months' observation. The preliminary results were reported by Garrison *et al.* (1952).

MATERIALS AND METHODS

All patients with clinical attacks of *vivax* malaria acquired in Korea who were admitted to the Station Hospitals between 16 July, 1951, and 1 May, 1952, were treated in rotation on one of the following regimens. After 1 May, 1952 the second regimen (pamaquine) was terminated; the other two were continued until 1 November, 1952. All follow-up was terminated 1 April, 1953.

Regimen A: Chloroquine—A total dose of 1.5 gm. chloroquine base (2.5 gm. of

* These studies were conducted at Fort Knox, Kentucky and Fort Benning, Georgia. Most of the primaquine was supplied gratuitously by Eli Lilly & Co. for which thanks are extended. This work was supported (in part) by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army.

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the diphosphate salt) was administered in 3 doses of 0.3 gm. each during the first 24 hours, followed by a single dose of 0.3 gm. daily for 2 days.

Regimen B: Pamaquine—Twenty-seven milligrams of pamaquine base (60 mg. of the naphthoate salt) were given daily in 3 equally divided doses for 14 consecutive days. In addition 1.5 gm. of chloroquine base were administered as in Regimen A.

Regimen C: Primaquine—Fifteen milligrams of primaquine base (26.4 mg. of the diphosphate salt) were given in a single daily dose for 14 consecutive days. A total dose of 1.5 gm. of chloroquine base was administered as in Regimen A.

Chloroquine was chosen for treatment of the control group because it eliminates parasitemia, fever and symptoms quickly, and because it is the standard suppressive agent used by the Armed Services. Because suppressive drugs are effective only against the erythrocytic forms of the parasite, they do not influence the relapse rate of the disease. Chloroquine was also administered with pamaquine and primaquine in regimens B and C because the curative drugs, which act against the persisting tissue stages of the *vivax* parasite, have too little effect on the blood stages to terminate the clinical attack effectively.

Pamaquine was included as the standard of reference (Regimen B) because this compound has been used extensively since its curative properties were first demonstrated in 1928 by Sinton and Bird. The dose of 27 mg. of pamaquine base (60 mg. of the naphthoate salt) was selected because that amount had effectively reduced the relapse rate of *vivax* malaria of Southwest Pacific origin in veterans of World War II (Most *et al.*, 1946). In planning this study it was assumed that veterans of the Korean conflict, having been exposed to infection for a shorter time than the veterans of World War II, would have less immunity than the latter and their infections would be further from natural termination, and consequently, would be less easily cured. Such proved not to be the case (*vide infra*).

A daily dose of 15 mg. of primaquine (Regimen C) was chosen because it is the largest daily dose that can be administered to Negroes for two weeks without close medical supervision (Hockwald *et al.*, 1952). This dose cured 76 per cent of experimental infections due to the Chesson (Southwest Pacific) strain of *vivax* malaria (Alving *et al.*, 1953).

The diagnosis of malaria due to *Plasmodium vivax* was established in each case by demonstrating parasites in the peripheral blood in two nonconsecutive smears.

All patients experienced fever greater than 101°F. (oral) before treatment was instituted. Patients were hospitalized for the duration of treatment and were observed daily for evidence of toxicity. A white blood cell count, hemoglobin concentration, and routine urinalysis were done when patients were admitted. These studies were repeated after one week for patients treated according to Regimen B or C, and at the end of therapy. Additional studies were performed as deemed necessary.

When treatment was completed the patients were discharged to duty and followed as out-patients at intervals of approximately six weeks. At each follow-up visit, patients were interviewed and a thick film of peripheral blood was examined for parasites. When relapses occurred patients were treated according to the

regimen originally employed. Only patients followed for more than four months after treatment are included in this report. The duration of observation after treatment is summarized in Table 1.

TABLE 1

Duration of follow-up after treatment in 1951 and 1952

DRUG REGIMEN	NUMBER OF CASES TREATED	MONTHS OF OBSERVATION		
		4 to 6	7 to 12	13 to 17
1951				
A. Chloroquine	258	43	169	46
B. Pamaquine plus chloroquine	272	43	191	38
C. Primaquine plus chloroquine	256	30	193	33
1952				
A. Chloroquine	97	78	19	
B. Primaquine plus chloroquine	92	67	25	

TABLE 2

Comparative relapse rates in 1951 and 1952

PATIENTS	DRUG REGIMEN IN TERMS OF BASE		
	Chloroquine 1.5 gm. in three days	Daily for 14 days	
		Pamaquine 27 mg.*	Primaquine 15 mg.*
1951			
Number treated†	258	272	256
Number that relapsed	71	2	0
Relapse rate (per cent)	27.5	0.7	0
1952			
Number treated†	97		92
Number that relapsed	66		0
Relapse rate (per cent)	67		0
Relapse rate for the two years			
Total number treated 1951–1952†	355	272	348
Total number that relapsed	137	2	0
Total relapse rate (per cent)	39	0.7	0

* Plus chloroquine 1.5 gm. (base) in three days.

† Only patients who were followed more than 4 months after treatment are included.

RESULTS

A total of 975 patients was treated and followed for more than four months after treatment. The clinical manifestations of the acute attack were rapidly

controlled by chloroquine. The relapse rate after treatment is shown in Table 2. One hundred and thirty-seven (39 per cent) of 355 patients treated with chloroquine alone relapsed after treatment. One hundred and thirty-three of the 137 patients who relapsed experienced a typical acute attack of malaria with fever and parasitemia; 4 had asymptomatic parasitemia without fever, which cleared spontaneously without treatment. The 133 patients who developed a febrile relapse were again treated with chloroquine alone. Twelve of the 133 (9 per cent) relapsed a second time, and two relapsed a third time.

Two (0.7 per cent) of the 272 patients treated with pamaquine relapsed after treatment. The relapse was a typical acute attack of malaria with fever and parasitemia in both instances. Relapse occurred 54 days after treatment in one patient; in the second patient relapse occurred 386 days (13 months) after treatment. This patient entered Korea in July 1950 and left in June, 1951. He was thus exposed to malaria during the transmission season of 1950 and 1951. We believe that he was infected first in 1950 and again in 1951, and that the attack in 1952 was the first late attack resulting from infection in 1951. Similar cases observed in patients treated with chloroquine only are described in the preceding paper (Hankey *et al.*, 1953). None of the 348 patients treated with primaquine relapsed after treatment.

In the course of these studies only minimal evidence of drug toxicity was observed. One patient treated with chloroquine alone developed mild generalized pruritus which subsided 4 days after the drug was stopped. A similar mild pruritus developed in one patient treated with chloroquine and primaquine. It disappeared after chloroquine was withdrawn, before therapy with primaquine was completed. A few of the patients treated with chloroquine plus pamaquine complained of mild crampy abdominal pain at some time during therapy. This was not of sufficient severity to warrant stopping the drug. There was no evidence of hemolytic anemia in any patient.

Approximately 15 per cent of the patients had a mild to moderately severe anemia (hemoglobin concentration of 6.4 to 12 gm. per 100 cc.) at the time treatment was instituted. In these patients the hemoglobin concentration increased during therapy as the acute attack was controlled. There was a slight but probably significant increase in leucocyte count at the end of therapy in some patients treated with pamaquine or primaquine. Methemoglobin determinations were not done; cyanosis was not observed.

DISCUSSION

The relapse rate after treatment with chloroquine alone (38 per cent) was considerably lower than the relapse rate of other strains of *vivax* malaria after treatment with equal or larger doses of chloroquine (Most *et al.*, 1946; Gordon *et al.*, 1947; Berliner *et al.*, 1948; Craige *et al.*, 1947; Edgcomb *et al.*, 1950; Alving *et al.*, 1953; and Cooper *et al.*, 1953). In the two hundred and fifty-eight patients treated between 15 July, 1951, and 31 December, 1951, the relapse rate was only 28 per cent, while the relapse rate in the 97 patients who were treated in 1952 was 67 per cent (Table 2). This difference in relapse rate came as a considerable surprise because it had been assumed on *a priori* grounds that the incidence of

relapse in 1951 would exceed the relapse rate in 1952 by a considerable margin. This assumption was made on the premise that men treated in 1951 had received heavier sporozoite infections than the men treated in 1952. Because Korean *vivax* malaria is temperate-zone type, a high percentage of the attacks treated in the United States were the result of infections acquired in Korea during the transmission season of April through September of the previous year. Nearly all of the patients treated in 1951 had been infected in Korea in the summer of 1950, and the patients treated in 1952 had been infected in Korea during the transmission season of 1951. Environmental conditions were almost ideal for heavy sporozoite inoculations when the troops were crowded into the small area of the Pusan Bridgehead in 1950. The troops were then intermingled with civilian refugees, who had swollen the population to such proportions that housing and sanitary facilities were woefully inadequate; effective mosquito control was virtually impossible. By the summer of 1951 the line of military operations had been stabilized near the 38° parallel, combat troops had much less contact with the native population, and the summer was unusually dry. Environmental conditions in the field in 1951 were much less favorable to intense mosquito transmission of malaria than had been the case in 1950.

A possible explanation for the apparent paradox in the relapse rates after chloroquine therapy, 1951 and 1952, may be found when one considers the natural history of Korean *vivax* malaria. The natural life span of the parasite, as encountered in returning military personnel, is somewhat less than two years (Hankey *et al.*, 1953). Because these studies were started on 15 July, 1951, the patients treated in 1951 were treated late in the course of their infection, when the parasite was approaching the end of its natural life span. Nearly all of the patients treated in 1952 were treated earlier in the course of their disease, i.e., during the spring and early summer near the onset of clinical activity following the period of long-term latency. It is our opinion that the difference in relapse rate in the two groups indicates that the "age of the disease" is a more important factor than the intensity of the sporozoite inoculation in determining the relapse rate after treatment of late attacks with noncurative drugs. Unfortunately, the data for the two years, when combined, does not overlap sufficiently to provide a definite answer to this question.

While clinically significant toxicity was not observed with either primaquine or pamaquine, this dose of pamaquine is potentially toxic to Negroes (Hockwald *et al.*, 1952). Since both curative regimens (27 mg. of pamaquine base and 15 mg. of primaquine base) were practically 100 per cent effective, the minimum amount of either drug needed to cure Korean *vivax* malaria cannot be determined from this study. The results indicate that naturally acquired *vivax* infections of Korean origin are cured more easily than the naturally acquired tropical *vivax* infections in veterans of World War II (Most *et al.*, 1946) or primary attacks of Chesson (tropical) strain which resulted from very heavy sporozoite inoculation (Craige *et al.*, 1947; Berliner *et al.*, 1948; Edgcomb *et al.*, 1950; Alving *et al.*, 1953, Cooper *et al.*, 1953). This greater susceptibility to curative action of the 8-aminoquinolines administered during *late* second summer activity appears to be an intrinsic property of temperate-zone strains of *vivax* infections. Thus Ruhe *et al.*

(1949) observed that the St. Elizabeth strain of *vivax* malaria, which is a temperate-zone type, presented only a mild challenge to curative drugs during late activity even though the sporozoite inocula the previous year had been extremely heavy. The St. Elizabeth infections were cured by 27 mg. of pamaquine base which, as we have shown, also cured Korean *vivax* malaria, but which was only partially effective against naturally acquired Southwest Pacific infections or experimentally induced Chesson strain infections. The St. Elizabeth studies included too few patients to allow conclusions to be drawn as to the relative importance of strain susceptibility, sporozoite dosage, or age of infection in determining the challenge to curative drugs; but, with the added data from the present studies the findings of Ruhe and his co-workers gain significance. It seems extremely unlikely that the sporozoite inocula of the Korean infections treated in 1952 approached the intensity of the St. Elizabeth infections, yet both were cured easily. It would appear therefore that the intensity of the original infection plays a minor role in determining the challenge to cure during late activity in strains of temperate zone origin. Present evidence is consistent with the view that temperate zone *vivax* infections, as exemplified by the Korean and St. Elizabeth strains, offer a weak challenge to curative drugs administered during late (second summer) clinical activity.

The effectiveness of curative drugs administered during early first summer clinical activity was not determined for the St. Elizabeth strain (Ruhe *et al.*, 1949) and can only be postulated from our results. Early attacks constituted only a small minority of the total number of clinical attacks treated (Hankey *et al.*, 1953); nevertheless, the fact that practically 100 per cent of the patients treated in 1951 and 1952 were cured must be accepted as presumptive evidence that both regimens, pamaquine as well as primaquine, exhibited considerable curative action during early as well as late clinical activity. Available facts do not justify precise evaluation of the effectiveness of curative drugs during early attacks, nor speculation as to the relationship between sporozoite dosage and challenge to cure presented by early primary attacks of either the St. Elizabeth or Korean strains of temperate zone *vivax* infections.

SUMMARY AND CONCLUSION

Fifteen milligrams of primaquine base administered daily for 14 days, in conjunction with standard chloroquine therapy (1.5 gm. base or 2.5 gm. chloroquine diphosphate in 3 days), constitutes the treatment of choice for the radical cure of *Plasmodium vivax* of Korean origin during late clinical activity. This therapeutic schedule may be safely continued on an ambulatory basis with only minimum medical supervision after acute symptoms have subsided.

Clinically significant toxicity was not observed with either primaquine or pamaquine, although in the dosage employed pamaquine is potentially toxic in a small percentage of Negroes.

REFERENCES

ALVING, A. S., ARNOLD, J., HOCKWALD, R. S., AND CLAYMAN, C. B., 1953. Unpublished data.
BERLINER, R. W., EARLE, D. P., JR., TAGGART, J. V., WELCH, W. J., ZUBROD, C. G., KNOWL-

TON, P., ATCHLEY, J. A., AND SHANNON, J. A., 1948. Studies on the chemotherapy of the human malarias. VII. The antimalarial activity of pamaquine, *J. Clin. Investigation* **XXVII** (Part 2): 108–113.

CLAYMAN, C. B., ARNOLD, J., HOCKWALD, R. S., YOUNT, E. H., JR., EDGCOMB, J. H., AND ALVING, A. S., 1952. Status of primaquine. 3. Toxicity of primaquine in Caucasians, *J.A.M.A.* **149:** 1563–1568.

COOPER, W. C., MYATT, A. V., HERNANDEZ, T., JEFFERY, G. M., AND COATNEY, G. R., 1953. Studies in human malaria. XXXI. Comparison of primaquine, isopentaquine, SN-3883, and pamaquine as curative agents against Chesson strain *vivax* malaria, *Am. J. Trop. Med. & Hyg.* **2:** 949–957.

CRAIGE, B., JR., JONES, R., JR., WHORTON, C. M., PULLMAN, T. N., ALVING, A. S., AND EICHELBERGER, L., 1947. Clinical standardization of pamaquin (Plasmochin) in mosquito-induced *vivax* malaria, Chesson strain. A preliminary report, *Am. J. Trop. Med.* **27:** 309–315.

EDGCOMB, J. H., ARNOLD, J., YOUNT, E. H., JR., ALVING, A. S., EICHELBERGER, L., JEFFERY, G. M., EYLES, D., AND YOUNG, M. D., 1950. Primaquine, SN-13272, A new curative agent in *vivax* malaria: A preliminary report, *J. Nat. Malaria Soc.* **9:** 285–292.

GARRISON, P. L., HANKEY, D. D., COKER, W. G., DONOVAN, W. N., JASTREMSKI, B., COATNEY, G. R., ALVING, A. S., AND JONES, R., JR., 1952. Status of primaquine. 2. Cure of Korean *vivax* malaria with pamaquine and primaquine, *J.A.M.A.* **149:** 1562–1563.

GORDON, H. H., DIEUAIDE, F. R., MARBLE, A., CHRISTIANSON, H. B., AND DAHL, L. K., 1947. Treatment of *Plasmodium vivax* malaria of foreign origin. A comparison of various drugs, *Arch. Int. Med.* **79:** 365–380.

HANKEY, D. D., JONES, R., JR., COATNEY, G. R., ALVING, A. S., COKER, W. G., GARRISON, P. L., DONOVAN, W. N., 1953. Korean *vivax* malaria. I. Natural history and response to chloroquine, *Am. J. Trop. Med. & Hyg.* **2:** 958–969.

HOCKWALD, R. S., ARNOLD, J., CLAYMAN, C. B., AND ALVING, A. S., 1952. Status of primaquine. 4. Toxicity of primaquine in Negroes, *J.A.M.A.* **149:** 1568–1570.

MOST, H., KANE, C. A., LAVIETES, P. H., LONDON, I. M., SCHROEDER, E. F., AND HAYMAN, J. M., 1946. Combined quinine-plasmochin treatment of vivax malaria: effect on relapse rate, *Am. J. M. Sc.* **212:** 550–560.

RUHE, D. S., COOPER, W. C., COATNEY, G. R., AND JOSEPHSON, E. S., 1949. Studies in human malaria. XV. The therapeutic action of pamaquine (Plasmochin) against St. Elizabeth strain *vivax* malaria, *Am. J. Hyg.* **49:** 367–373.

SINTON, J. A., AND BIRD, W., 1928. Studies in malaria with special reference to treatment. Part IX. Plasmochin in the treatment of malaria, *Indian J. M. Research* **16:** 159–177.

KOREAN *VIVAX* MALARIA

III. Curative Effect and Toxicity of Primaquine in Doses from 10 to 30 mg. Daily*

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It is the purpose of this paper to present the results of a study designed to determine the optimum dose of primaquine for the curative treatment of *vivax* malaria of Korean origin. Data available at the time these studies were initiated indicated that 22.5 mg. of primaquine base was the minimum dose uniformly effective in curing human infections of the Chesson (tropical) strain of *Plasmodium vivax* (Edgcomb *et al.*, 1950). Because there was no information on the response of Korean *vivax* malaria to curative drugs, four different 14-day regimens, with daily doses of 10, 15, 20, and 30 mg. were investigated. Later in the course of the study, when it became apparent that a daily dose of 30 mg. was excessive, that regimen was discontinued, and a new one employing 20 mg. of primaquine daily for 7 days was substituted.

MATERIALS AND METHODS

Nine hundred and sixty-three patients with *vivax* malaria acquired in Korea were treated at the time of a late acute attack and followed for more than four months thereafter. The diagnosis was established by demonstrating parasites in each of two nonconsecutive smears of peripheral blood. Each patient experienced fever greater than 101°F. before treatment was instituted.

Patients were treated in rotation according to one of the following regimens: (1) chloroquine 1.5 gm. (base) in 3 days (3 doses of 0.3 gm. were given during the first day plus a single dose of 0.3 gm. on each of the next two days); (2) chloroquine, administered as above, plus primaquine at 10, 15, 20, and 30 mg. (base) daily. Primaquine was started with the first dose of chloroquine and continued for 7 or 14 consecutive days. When the daily dose of primaquine was 10 or 15 mg. it was administered in a single tablet; when the daily dose was 20 or 30 mg., 10 mg. were administered twice or three times daily. Each dosage regimen of prima-

* This study was conducted at Camp Breckenridge, Kentucky, Fort Meade, Maryland, Fort Dix, New Jersey, and in the Naval Hospital at Camp Lejeune, North Carolina, between 1 August 1951 and 1 January 1953. Most of the primaquine was supplied gratuitously by Eli Lilly & Co. for which thanks are extended. This work was supported (in part) by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army.

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quine was studied simultaneously in at least two different hospitals. The laboratory studies and follow-up procedures were identical with those described in the preceding papers. The methemoglobin concentration was determined by the method of Evelyn and Malloy (1938).

TABLE 1

Treatment of Korean vivax *malaria with primaquine in 1951 and 1952*

PATIENTS	DRUG REGIMEN				
	Chloroquine 1.5 gm. (base) in three days	Primaquine (base) daily for 14 days			
		10 mg.*	15 mg.*	20 mg.*	30 mg.*
Number treated†	334	157	182	131	41
Number that relapsed	146	4	2	0	0
Relapse rate (per cent)	43.8	2.5	1	0	0

* Plus chloroquine 1.5 gm. (base) in three days.

† Only patients who were followed more than 4 months after treatment are included.

TABLE 2

Treatment of Korean vivax *malaria with primaquine in 1951*

PATIENTS	DRUG REGIMEN				
	Chloroquine 1.5 gm. (base) in three days	Primaquine (base) daily for 14 days			
		10 mg.*	15 mg.*	20 mg.*	30 mg.*
Number treated†	119	53	82	89	18
Number that relapsed	35	1	1	0	0
Relapse rate (per cent)	30	2	1	0	0

* Plus chloroquine 1.5 gm. (base) in three days.

† Only patients who were followed more than 4 months after treatment are included.

RESULTS

A. *Curative effect*

Fourteen-day regimens. The effect of a daily dose of 10 to 30 mg. of primaquine given for 14 consecutive days on the relapse rate is summarized in Table 1. A total of 334 patients was treated with chloroquine alone and followed for more than four months after treatment. One hundred and forty-six (43.8 per cent) of these patients relapsed at least once after treatment. Four (2.5 per cent) of 157 patients treated with 10 mg. of primaquine relapsed, and 2 patients (1 per cent) treated with 15 mg. of primaquine relapsed. None of the patients treated with 20 or 30 mg. of primaquine daily relapsed after treatment.

Because this study was started in August, 1951, the patients treated in 1951 were treated late in the course of the infection, when the parasite was approaching the end of its life span. Seventy-five per cent of the patients treated in 1952 were treated before the first of July, and all were treated before the first of August. Hence the patients treated in 1952 were treated earlier in the course of their

infection, at the time of onset of clinical activity after the latent interval. The relapse rate after treatment in patients treated in 1951 is shown in Table 2. In this group of patients, the relapse rate after treatment with chloroquine alone was only 30 per cent. One patient treated with 10 mg. and one patient treated with 15 mg. of primaquine daily relapsed after treatment.

TABLE 3

Treatment of Korean vivax *malaria with primaquine in 1952*

PATIENTS	DRUG REGIMEN				
	Chloroquine 1.5 gm. (base) in three days	Primaquine (base) daily for 14 days			
		10 mg.*	15 mg.*	20 mg.*	30 mg.*
Number treated†	215	104	102	42	23
Number that relapsed	111	3	1	0	0
Relapse rate (per cent)	51.6	3	1	0	0

* Plus chloroquine 1.5 gm. (base) in three days.

† Only patients who were followed more than 4 months after treatment are included.

TABLE 4

Treatment of Korean vivax *malaria with primaquine administered daily for 7 days*

PATIENTS	DRUG REGIMEN	
	Chloroquine 1.5 gm. (base) in three days	Primaquine 20 mg. (base) daily for 7 days*
Number treated†	70	68
Number that relapsed	36	2
Relapse rate (per cent)	51	3

* Plus chloroquine 1.5 gm. (base) in 3 days.

† Only patients who were followed more than 4 months after treatment are included.

The relapse rate in patients treated in 1952 is shown in Table 3. In this group the relapse rate after treatment with chloroquine alone was 51.6 per cent. Three patients relapsed after treatment with 10 mg.; the relapse was typical, fever and parasitemia in each case. One patient who was treated with 15 mg. of primaquine developed a febrile illness while on leave 71 days after treatment. He was treated with quinine; parasites were not demonstrated. This patient has been well for 131 days since that episode.

None of the patients treated with 20 or 30 mg. of primaquine daily relapsed after treatment.

Seven-day regimen. During the spring and summer of 1952 an additional regimen of 20 mg. of primaquine daily for 7 days was studied in order to determine whether a shorter period of treatment was equally effective. Results are summarized in Table 4. In this study, 36 patients (51 per cent) treated with chloroquine alone relapsed at least once after treatment. Two (3 per cent) of the 68

patients treated with 20 mg. of primaqunie daily developed typical relapses with fever and parasitemia 51 and 68 days, respectively, after treatment.

B. *Toxicity*

The toxic effects produced by primaquine and the number of patients in whom they were observed are shown in Table 5. In the group of patients treated with 10 mg. of primaquine daily, one patient developed fairly marked cyanosis near the end of treatment. He was found to have 1.4 gm. of methemoglobin on the thirteenth day of treatment. He was asymptomatic for malaria at that time; the cyanosis cleared within 3 days after treatment was completed. Approximately 2 per cent of patients treated with 15 mg. and 6 per cent treated with 20 mg. of

TABLE 5

Toxicity of primaquine in patients treated in 1951 and 1952

DAILY DOSE	NUMBER OF PATIENTS	PER CENT WITH CYANOSIS	PER CENT WITH ABDOMINAL PAIN	LEUCOPENIA	ACUTE HEMOLYTIC ANEMIA
mg.					
10	157	0.5	0	0	0
15	181	2	4	0	0
20	131	6	7	0	1*
30	41	20	24	0	0

* In one of 14 Negroes treated.

primaquine daily for 14 days were thought to have had some degree of cyanosis during treatment, and 8 (20 per cent) of the 41 patients who received 30 mg. of primaquine daily were cyanotic before treatment was completed. In these 8 patients the concentration of methemoglobin was 8 to 14 per cent of total hemoglobin on the last day of treatment. Cyanosis cleared promptly in every case after treatment was completed. Intermittent, crampy abdominal pain was noted at sometime during therapy by 10 (24 per cent) of the 41 patients treated with 30 mg. of primaquine daily. The pain was never severe enough to interfere with the patient's activity on the ward, or to warrant stopping the drug. In patients treated with 15 or 20 mg. daily this symptom was so mild as to be of little significance. There was no evidence of depression of white blood cell count in any individual. In a small number of patients who were mildly leucopenic when treatment was instituted, the white blood cell count returned to normal levels during treatment.

One patient, a 19-year old Negro male, who was treated with 20 mg. of primaquine daily for 14 days, developed a moderately severe hemolytic anemia. The hemoglobin concentration fell from 14.6 gm. at the time treatment was instituted to 9.4 gm. on the eighth day of therapy and the red blood cell count fell from 4.4 million to 3.2 million. Laboratory studies at this time revealed an increase in delayed-reacting bilirubin and there was hemoglobinuria of moderate degree. Tests of the red blood cells for sickling and fragility to hypotonic saline were normal and the bone marrow was moderately hypercellular with increased

erythropoiesis. The patient was asymptomatic at this time. The drug was withdrawn on the eighth day of treatment, and the patient was given enough sodium bicarbonate by mouth to keep his urine alkaline for the next four days. Two days after treatment was stopped the hemoglobin concentration was 8.2 gm. per 100 ml. The patient was given 1,000 cc. of fresh whole blood at that time, and the hemoglobin concentration and red blood cell count returned to normal levels and remained normal during the next three months. This patient was one of 14 Negroes treated with 20 mg. of primaquine daily for 14 days. There was no evidence of hemolysis in the other 13 Negroes. Eight other Negroes were treated with 15 mg. of primaquine daily and nine others received 10 mg. In none was there evidence of hemolysis during treatment. There were no Negroes in the group treated with 30 mg. of primaquine daily.

DISCUSSION

It is apparent that all doses of primaquine used in this study, when administered with chloroquine for the treatment of late attacks of *Plasmodium vivax* of Korean origin, will eradicate the disease in nearly all patients. The difference in relapse rate after treatment with 10 mg., 15 mg., and 20 mg., daily, has little statistical significance. While the results suggest that a daily dose of 10 mg. for 14 days or 20 mg. for 7 days is the minimum dose that will cure Korean *vivax* malaria in nearly all patients, this has not been established with certainty. It is possible that a smaller dose would be equally effective. It has been demonstrated that a daily dose of 10 mg. of primaquine for 14 days is adequate for the curative treatment of *vivax* malaria of Korean origin and the period of treatment may be shortened to 7 days by increasing the daily dose to 20 mg. These results confirm and extend the conclusion expressed in the preceding paper (Alving *et al.*, 1953) that naturally acquired *vivax* infections of Korean origin are more susceptible than experimentally induced Chesson strain *vivax* infections to the curative action of primaquine.

The acute hemolytic anemia that occurred in one Negro during treatment with 20 mg. of primaquine was undoubtedly due to the drug. Hockwald *et al.* (1952) have demonstrated that a daily dose of 30 mg. of primaquine will produce severe hemolytic anemia in approximately 5 per cent of Negroes. They reported that a daily dose of 15 mg. of primaquine for 14 days was well tolerated by five Negro volunteers in whom a daily dose of 30 mg. of primaquine had produced severe intravascular hemolysis. In our patient the degree of anemia produced by 20 mg. of primaquine daily was equal in severity to that produced by 30 mg. of primaquine daily in patients studied by Hockwald and his co-workers.

SUMMARY

A study of the therapeutic effectiveness and toxicity of primaquine in daily doses of 10, 15, 20, and 30 mg. (base) daily for 7 or 14 days, administered concurrently with 1.5 gm. (base) of chloroquine in 3 days, at the time of an acute attack of *vivax* malaria of Korean origin is reported. The relapse rate in patients treated with chloroquine alone was 43.8 per cent. The relapse rate was reduced

to less than 3 per cent by all regimens of primaquine used. These results indicate that a daily dose of 10 mg. of primaquine administered for 14 consecutive days is adequate for the curative treatment of *vivax* malaria of Korean origin. A daily dose of 20 mg. administered for 7 days was effective in reducing the relapse rate.

No significant toxicity was observed with doses of 10 and 15 mg. daily. A daily dose of 20 mg. of primaquine produced acute severe intravascular hemolysis in one of 14 Negroes. This dose was well tolerated by all other patients. Mild abdominal pain and cyanosis due to methemoglobinemia occurred in approximately one-fourth of the white patients treated with 30 mg. of primaquine daily, but these effects were not severe enough to interfere with normal activity.

REFERENCES

ALVING, A. S., HANKEY, D. D., COATNEY, G. R., JONES, R., JR., COKER, W. G., GARRISON, P. L., AND DONOVAN, W. N., 1953. Korean *vivax* malaria. II. Curative treatment with pamaquine and primaquine, *Am. J. Trop. Med. & Hyg.* **2**: 970–976.

EDGCOMB, J. H., ARNOLD, J., YOUNT, E. H., ALVING, A. S., AND EICHELBERGER, L., 1950. Primaquine: SN-13,272, a new curative agent in *vivax* malaria: a preliminary report, *J. Nat. Malaria Soc.* **9**: 285–292.

EVELYN, K. A., AND MALLOY, H. I., 1938. Microdetermination of oxyhemoglobin, methemoglobin and sulfhemoglobin in a single sample of blood, *J. Biol. Chem.* **126**: 655–662.

HOCKWALD, R. S., ARNOLD, J., CLAYMAN, C. B., AND ALVING, A. S., 1952. Toxicity of primaquine in negroes, *J.A.M.A.* **149**: 1568–1570.

KOREAN *VIVAX* MALARIA

IV. Curative Effect of 15 Milligrams of Primaquine Daily for 7 Days*

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Studies by Edgcomb *et al.* (1950), by Garrison *et al.* (1952), and Alving *et al.* (1953) have shown that primaquine in 15-milligram doses for 14 days will produce radical cure of nearly all cases of Korean *vivax* malaria. This report presents the results of a study of the effect of 15 mg. of primaquine daily for 7 days.

MATERIALS AND METHODS

The methods used in this study were similar to those described in the preceding papers of this series. All patients admitted to the station hospital with

TABLE 1

Effect of giving 15 mg. primaquine daily for 7 days against Korean vivax *malaria*

PATIENTS	DRUG REGIMEN	
	Chloroquine 1.5 gm. (base) in 3 days	Primaquine (base) 15 mg./day for 7 days plus chloroquine 1.5 gm. (base)
Number treated and followed more than 90 days	46	31
Number that relapsed	23	1
Relapse rate (per cent)	50	3

vivax malaria between 15 June, 1952, and 1 October, 1953, were treated with 1.5 gm. of chloroquine (base) in 3 days. Three doses of 0.3 gm. were given during the first 24 hours, and a single dose of 0.3 gm. was given on each of the next two days. Half of the patients was given 15 mg. (base) of primaquine daily for 7 days in addition to chloroquine. The patients were treated alternately as they were admitted, with chloroquine alone, or chloroquine plus primaquine. Only those patients who were followed for more than 90 days after treatment are included in this report.

RESULTS

The relapse rate after treatment with chloroquine alone and chloroquine plus primaquine is shown in Table 1. Twenty-three (50 per cent) of the 46 patients

* This study was conducted at Camp Breckenridge, Kentucky. Most of the primaquine was supplied gratuitously by Eli Lilly & Co. for which thanks are extended. This work was supported (in part) by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army.

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treated with chloroquine alone and followed for more than 90 days relapsed at least once. Only one (3 per cent) of the 31 patients who received 15 mg. of primaquine daily for 7 days relapsed after treatment. This patient developed a typical febrile relapse with parasitemia 49 days after treatment. No untoward effects of treatment occurred in any patient in this study.

DISCUSSION

While it is obvious that 15 mg. of primaquine produced a highly significant reduction in the relapse rate, results must be interpreted with caution for the following reasons: (1) the total number of patients studied was relatively small; (2) the duration of follow-up in approximately one-third of the patients in each group was only 90 to 119 days. Analysis of the relapse rate of Korean *vivax* malaria after treatment with chloroquine alone indicates that only two-thirds of the patients who ultimately relapse will do so within 90 days after treatment while 95 per cent of relapses occur within 120 days (Hankey *et al.*, 1953); (3) studies of experimental *vivax* malaria in prisoner-volunteers indicate that when primaquine is administered at near the minimum effective dose, it may prolong the latent interval after treatment in patients who are not cured (Edgcomb *et al.*, 1950).

In spite of these reservations in the interpretation of results, the curative activity of primaquine when administered in a daily dose of 15 mg. for only 7 days is sufficiently great to warrant recommending its use as a substitute for the standard regimen of 14 days, when conditions are such as to make it impractical to administer the drug for the longer period. Although discussed in an earlier paper (Alving *et al.*, 1953), it is important to re-emphasize that radical cure of *vivax* malaria in Korean veterans is easily achieved with curative drugs. For the present, therefore, the recommendation must be limited to autochthonous infections of Korean origin.

SUMMARY

In a study of the curative effect of 15 mg. of primaquine daily for 7 days against late attacks, the relapse rate in patients with Korean *vivax* malaria was reduced from 50 per cent in patients treated with chloroquine to 3 per cent in patients treated with chloroquine and primaquine. The minimum follow-up in any patient was 3 months.

REFERENCES

ALVING, A. S., HANKEY, D. D., COATNEY, G. R., JONES, R., JR., COKER, W. G., GARRISON, P. L., DONOVAN, W. N., 1953. Korean *vivax* malaria. II. Curative treatment with pamaquine and primaquine, *Am. J. Trop. Med. & Hyg.* **2:** 970–976.

EDGCOMB, J. H., ARNOLD, J., YOUNT, E. H., ALVING, A. S., AND EICHELBERGER, L., 1950. Primaquine: SN-13272, a new curative agent in *vivax* malaria: a preliminary report, *J. Nat. Malaria Soc.* **9:** 285–292.

GARRISON, P. L., HANKEY, D. D., COKER, W. G., DONOVAN, W. N., JASTREMSKI, B., COATNEY, G. R., ALVING, A. S., JONES, R., JR., 1952. Status of primaquine. 2. Cure of Korean *vivax* malaria with pamaquine and primaquine, *J.A.M.A.* **149:** 1562–1563.

HANKEY, D. D., JONES, R., JR., COATNEY, G. R., ALVING, A. S., COKER, W. G., GARRISON, P. L., AND DONOVAN, W. N., 1953. Korean *vivax* malaria. I. Natural history, clinical manifestations, and response to chloroquine, *Am. J. Trop. Med. & Hyg.* **2:** 958–969.

KOREAN *VIVAX* MALARIA

V. Cure of the Infection by Primaquine Administered during Long-term Latency*

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The phenomenon of long-term latency exhibited by certain strains of *vivax* malaria was recognized as early as 1897 when Thayer offered his speculations to account for it. During the next half century little progress was made toward locating the persistent forms responsible for the late attacks and then within a relatively short period these forms (exoerythrocytic) were actually demonstrated (see Huff, 1947; Shortt, 1951). Prior to their actual demonstration there was strong presumptive evidence (Cooper *et al.*, 1947, 1949) that attacks following an extended latent period resulted from fixed tissue parasites and not from an "activated" low-grade parasitemia. Working on this premise White *et al.* (1948) were able to cure St. Elizabeth strain *vivax* malaria in patients treated with pentaquine, an 8-aminoquinoline, during latency, and recommended such an elective treatment for the cure of *vivax* malaria.

A similar but more extensive study of the curative properties of primaquine, another 8-aminoquinoline, administered during latency, was made possible by the large scale toxicity studies undertaken in the summer of 1951 at Fort Benning, Georgia and Fort Knox, Kentucky which established the relative safety of administering 15 mg. of primaquine daily for 14 days to troops on full duty. Later in 1951 Alving *et al.* (1952) demonstrated that it was safe and feasible to administer the drug to men on shipboard while crossing the Pacific. On the basis of these studies mass therapy of Korean returnees with primaquine was recommended for the eradication of persisting tissue parasites. The study reported here was designed to evaluate the effectiveness of primaquine against Korean *vivax* malaria when administered during long-term latency.

MATERIAL AND METHODS

During the last week of March and the first two weeks of April, 1952, all Korean veterans at four army posts were interviewed. A group of 625 soldiers,

* These studies were conducted at Fort Benning, Georgia, Fort Knox, Kentucky, Fort Dix, New Jersey, and Camp Breckenridge, Kentucky. Most of the primaquine was supplied gratuitously by Eli Lilly & Co. for which thanks are extended. This work was supported (in part) by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army.

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who had been exposed to malaria in Korea during the summer of 1951 and who had returned to this country before January, 1952, was selected for participation in the study. No man who had ever had a clinical attack of malaria was accepted; Negroes were excluded because of their high racial immunity to *vivax* malaria.

Early in April, 1952 the study group at each post was divided by lot into two sections. The men in section A received 15 mg. of primaquine daily; those in section B, the control group, received a placebo containing lactose, outwardly indistinguishable from the medication given to those in section A. Each man was given a single pill each morning for 14 consecutive days; all were on full duty during treatment. A thick film of peripheral blood was prepared and examined before treatment in order to exclude the presence of parasitemia.

TABLE 1

Effect of primaquine treatment during long-term latency of Korean vivax *malaria*

SUBJECTS	TREATMENT REGIMEN	
	Placebo	Primaquine 15 mg./day for 14 days
Number treated	331	294
Number developed malaria	58	0
Per cent developed malaria	17.5	0

All men were held at their respective posts by a Department of Army order for six months after treatment. Except in unusual circumstances, no man was allowed more than 7 consecutive days' leave during the period of study.

All patients on the post with malaria were admitted to the post hospital for treatment. The diagnosis in every case was established by demonstrating parasites in the peripheral blood in two nonconsecutive smears. At the end of September, six months after treatment, all men were interviewed and questioned in detail regarding the occurrence of any illness which might have been malaria.

RESULTS

A total of 625 men participated in the study and all were treated during April. Two hundred ninety-four men (294) were treated with primaquine and 331 received the placebo (Table 1). During the six-months' period of observation 53 men who received the placebo developed clinical malaria and were hospitalized. Five other men, who had received the placebo, gave a clear-cut history of malaria attacks while on leave, and were treated elsewhere. Thus 58 (17.5 per cent) of 331 men, treated with a placebo, developed malaria. None of the 294 men who were treated with primaquine developed malaria during the six-months' period following treatment.

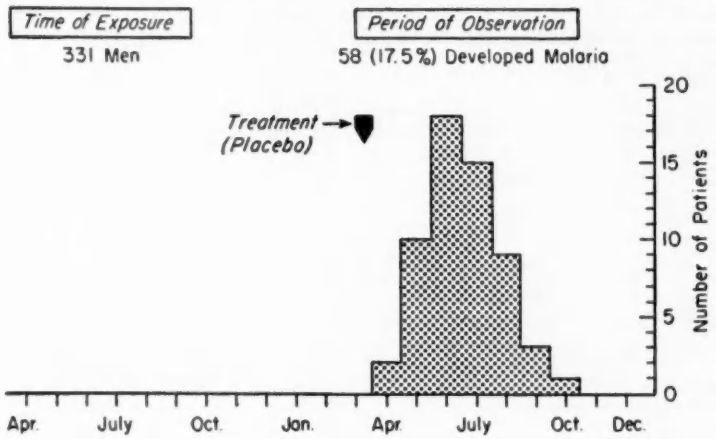
The attacks in the control group began during the last week of April, reached a maximum in June and July, and diminished thereafter. One man developed his first attack on 29 October, 1952. He entered Korea in July, 1950 and left in July, 1951. The period of latency in this patient was at least 15 months.

DISCUSSION

In our earlier studies patients with acute attacks of malaria were given primaquine concomitantly with chloroquine; acute attacks were controlled by the latter drug, and cures were brought about by the former. In these studies primaquine was administered alone during latency, when circulating erythrocytic parasites are absent, and again cure was achieved, showing that it is not necessary to administer suppressive drugs with primaquine in order to obtain cure of Korean *vivax* infections. Hence, mass primaquine therapy of troops returning from Korea should be highly effective in preventing subsequent attacks.

Figure 1

TIME OF THE FIRST LATE ATTACK OF KOREAN VIVAX MALARIA IN THE 58 PATIENTS IN THE CONTROL GROUP WHO DEVELOPED MALARIA



The pattern of clinical activity observed in the control group (Figure 1) confirms many of the observations on the natural history of Korean *vivax* malaria as detailed in the first paper of this series (Hankey *et al.*, 1953). In that paper it was pointed out that the pattern of clinical activity during 1951 was altered because many of the subjects received a variable amount of suppressive chloroquine during the spring and early summer of 1951. Because the patients in the current study had not received suppressive treatment for many months, the pattern of clinical attacks in the placebo-treated subjects portrays the natural seasonal distribution of late clinical activity.

SUMMARY

In a study of the curative activity of primaquine administered during long-term latency of Korean *vivax* malaria, 294 soldiers, exposed to infection in the summer of 1951 were treated early in April, 1952 with 15 mg. of primaquine daily for 14 days. A control group of 331 men was given a placebo. None of the

men treated with primaquine developed malaria during the next six months; 17.5 per cent of those given the placebo did develop malaria during the same period.

REFERENCES

ALVING, A. S., ARNOLD, J. AND ROBINSON, D. H., 1952. Status of primaquine. 1. Mass therapy of subclinical *vivax* malaria with primaquine, *J.A.M.A.* **149:** 1558–1562.

COOPER, W. C., COATNEY, G. R., AND RUHE, D. S., 1947. Studies in human malaria. V. Homologous strain superinfection during latency in subjects with sporozoite-induced *vivax* malaria (St. Elizabeth strain), *Am. J. Hyg.* **46:** 141–148.

COOPER, W. C., RUHE, D. S., AND COATNEY, G. R., 1949. Studies in human malaria. XVI. Results of massive subinoculation during latency from patients infected with St. Elizabeth strain *vivax* malaria, *Am. J. Hyg.* **50:** 189–193.

HANKEY, D. D., JONES, R., JR., COATNEY, G. R., ALVING, A. S., COKER, W. G., GARRISON, P. L., AND DONOVAN, W. N., 1953. Korean *vivax* malaria. I. Natural history, clinical manifestations, and response to chloroquine, Am. J. Trop. Med. & Hyg. **2:** 958–969.

HUFF, C. G., 1947. Life cycle of malarial parasites, *Ann. Rev. Microbiol.* pp. 43–60.

SHORTT, H. E., 1951. Life-cycle of mammalian malaria parasite, *Brit. Med. Bull.* **8:** 7–10.

THAYER, W. S., 1897. *Lectures on malarial fevers*, D. Appleton & Co., New York.

WHITE, W. C., COOPER, W. C., COATNEY, G. R., CULWELL, W. B., LINTS, H. A., AND YOUNG, M. D., 1948. Studies in human malaria. XXI. The cure of St. Elizabeth strain *vivax* malaria by pentaquine-quinine, administered during acute attacks or during latency, *J. Nat. Malaria Soc.*, **7:** 316–321.

A CLINICAL STUDY OF PRIMAQUINE (S. N. 13,272) IN THE TREATMENT OF MALARIA AMONG THE MISKITO INDIANS OF NICARAGUA[1]

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Since 1948, primaquine, a close relative of pamaquine, the historically important curative antimalarial agent, has been the subject of intensive clinical study at Stateville Penitentiary (Edgcomb *et al.*, 1950; Hockwald *et al.*, 1952; Clayman *et al.*, 1952). More recently primaquine has been used on a large scale among military personnel (Alving *et al.*, 1952). These studies have shown that primaquine is safe and practical for mass prophylaxis and therapy of malaria in nonimmune Caucasians and Negroes in a nonendemic area, and as such is the drug of choice for curative treatment of relapsing malaria (Alving *et al.*, 1953).

There remain, however, two very important problems: (1) testing the tolerance of tropical native peoples to primaquine and (2) measuring the response of field infections to treatment with that drug. These problems involve several factors not present in previous studies. Very few tropical populations are well nourished. Many inhabitants of endemic areas have suffered from chronic malaria for years and many have carried other parasites which have added to their general debility. This study was arranged to evaluate the effect of these factors on the tropical native's tolerance to primaquine and to attempt a therapeutic evaluation of primaquine in an endemic area. It is recognized, however, that evaluation of a curative drug is never very satisfactory in an endemic area where immunity and reinfection cannot be closely controlled.

REGION AND PEOPLES INVOLVED IN THE STUDY

The general area encompassed by this study was along the lower Rio Coco separating Honduras from Nicaragua. This is the largest river in Central America and lies about 15° North latitude. At mid-river it is only 100 feet above sea level. The terrain of the entire region, from the Patucca river in Honduras to about the level of the Rio Grande in Nicaragua, is composed of low gravel and clay ridges intermittently covered by dense forest and open grasslands. Inasmuch as the most fertile areas are closely adjacent to the major rivers, the bulk of the

[1] This study was supported by Army Contract No. DA-49-007-MD-20 between the University of Chicago and the Medical Research and Development Board, Office of the Surgeon General, Department of the Army. It was carried out in Nicaragua at the Hospital Ruth C. S. Thaeler, de la Mision Morava, Bilwaskarma, Nicaragua, on invitation of the Nicaraguan government. Part of the publication costs was borne by Winthrop-Stearns, Incorporated, New York.

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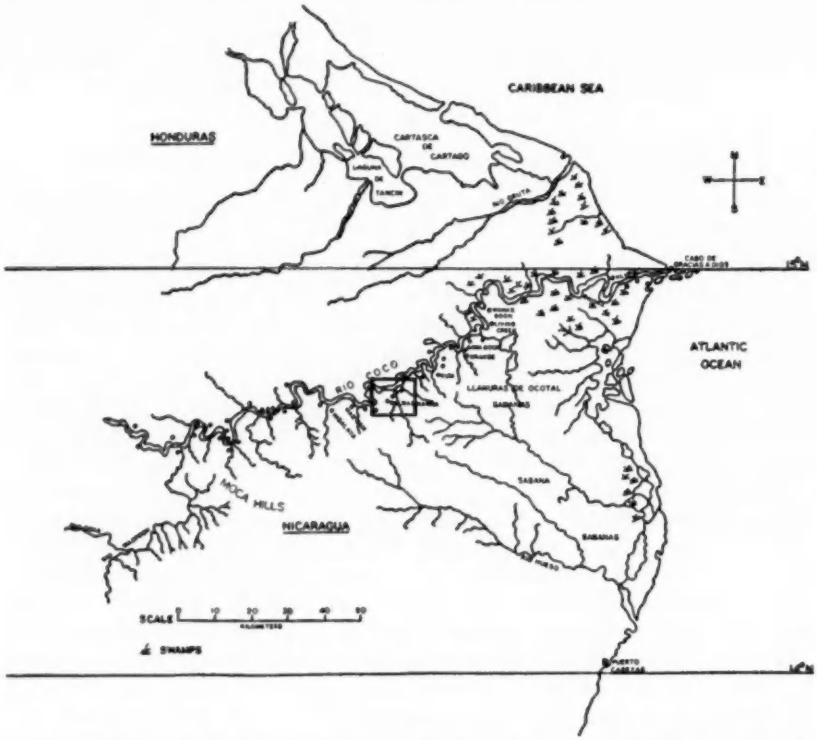

FIG. 1. Sketch of the Coco river basin used in study of primaquine therapy for malaria. The initial malaria survey of 1951 was made in the 5 villages included in the square. All positive cases discovered in this area received either curative primaquine or suppressive chloroquine treatment. All villages outside the square were surveyed in 1952. No special malaria therapy administered in this study. All villages indicated on the map by (●) had residual DDT spray beginning in late 1950.

TABLE 1

Blood types found in survey of Eastern Nicaraguan natives (Miskito Indians with Caucasian and Negro blood)

RACE	A	B	A-B	O	TOTALS
Miskito (pure)	1	1	0	59	61
Miskito (white)	5	2	0	10	17
Miskito (Negro)	2	0	0	12	14
Totals	8	3	0	81	92

population resides in numerous villages strung along the course of the rivers. The limit to the tillable soil restricts the population of each village to from 150 to 300 people. The villages are usually four to five miles apart (Figure 1). These

villages had been sprayed twice with residual DDT prior to the beginning of this study and it was expected that malaria transmission would be reduced considerably.

The inhabitants of this region are mostly Indians of the Miskito race. Fifty per cent of them have Negro ancestors and a few have Caucasian ancestors. It is interesting that of the pure-blooded Indians examined, all but two had type O blood (Table 1). With their dark skin and admixture of Negro ancestry, it might be expected that these people would be susceptible to a severe form of hemolytic anemia previously described in American Negroes (Hockwald *et al.*, 1952).

DISEASE DISTRIBUTION AND EPIDEMIOLOGY OF REGION

Of 1,113 people examined in the initial survey in May, June and July, 1951 (Figure 1), 29 per cent were found to have demonstrable parasites on thick blood smear examinations. The search for parasites involved examining approximately 0.1 cu. mm. of blood. This examination will reveal parasites only if they appear in concentrations of 10 per cu. mm. or more. Approximately 20 per cent

TABLE 2

Distribution by age of individuals found positive for malaria in a survey of Eastern Nicaragua natives in 1951

AGE GROUP	NUMBER SURVEYED	NUMBER POSITIVE	PER CENT POSITIVE
Up to 2 years	61	20	32.8
2 to 7 years	242	87	35.9
7 to 13 years	248	71	28.6
Over 13 years	562	152	27.0
Totals	1,113	330	29.7

of people with patent infections were febrile at the time they were discovered. Except for 18 cases infected with *P. falciparum*, and 3 cases infected with both *P. vivax* and *P. falciparum*, all cases were infected with *P. vivax* alone. The age distribution of malaria in the population seemed to favor the pre-adolescent child (Table 2). Along with malaria, the subjects of this study had associated parasitic infestations which could have modified their reaction to the drug. Of those treated, 93.7 per cent had intestinal parasites; 72.6 per cent were infested with *Necator americanus*. These infestations are probably responsible for the high incidence of anemia ranging down to 4.0 Gms. per cent (Table 3). Because beans are a dietary staple and contain relatively large amounts of iron, it is likely that the blood-loss anemias were moderated as a result. In addition to *Necator americanus*, *Ascaris lumbricoides* was found in 83.3 per cent of all patients (Table 4). About 65 per cent of all patients had active lesions of pinta; 5 per cent had yaws. About 30 per cent of all bloods examined demonstrated cold agglutination; 75 per cent had positive Kahn tests.

Although the incidence of malaria in May, June and July of 1951 could be fairly readily determined, there is no data pertaining to the seasonal variation

TABLE 3

Incidence of anemia found in survey of Eastern Nicaraguan natives suffering from malaria (chiefly P. vivax)

HEMOGLOBIN GMS. PER 100 CC	NUMBER	PERCENTAGE (OF PERSONS EXAMINED)
Up to 4.0	1	0.3
4.1 to 8.0	49	16.0
8.1 to 12.0	187	61.1
12.1 and up	69	22.6
Totals	306	100.0

TABLE 4

The incidence and type of intestinal parasites complicating the malaria cases treated in this study

SPECIES	PER CENT
Trichuris trichiura (alone)	1.5
Strongyloides stercoralis (alone)	1.1
Necator americanus (alone)	4.8
Ascaris lumbricoides (alone)	8.9
Ascaris and Necator	18.3
Ascaris and Trichuris	9.6
Ascaris, Necator and Trichuris	46.5
Trichuris and Necator	3.0
Total	93.7
Percentage of the above having Necator americanus alone or with other infestation	72.6
Percentage of the above having Ascaris lumbricoides alone or with other infestation	83.3

TABLE 5

Monthly rainfall in inches for Bilwaskarma on the Rio Coco in Eastern Nicaragua during the years 1951 and 1952 (incomplete data)

MONTH	1951	1952
January		4.56
February		2.05
March		1.47
April		4.99
May	3.03	7.52
June	17.42	20.56
July	22.66	18.05
August	10.90	10.83
September	9.54	5.15
October	9.85	12.37
November	12.33	6.62
December	7.23	

of the disease. Measurements of rainfall show a marked variation from month to month. In general, the last 6 months of the year are considered the rainy season as shown in Table 5, the data for which were collected during the course of this study. The major vector was not established.

PROCEDURES OF STUDY

From repeated surveys a total of 321 adults and children with malaria were found, chiefly in 5 preselected villages (Figure 1), and treated with a combination of primaquine and chloroquine. Of these, one patient had *P. falciparum*—all others had *P. vivax*. All patients treated were observed daily during treatment. Early in the course of the study treatment was done in the hospital. However, as experience with the drug accumulated, a number of patients were treated on an ambulatory basis with daily clinical and laboratory observation.

Before therapy, all patients were weighed in order to calculate the dose of drug. In addition, blood pressures, hemoglobin determinations, red and white blood cell counts, complete urinalyses, and stool examinations were done on all patients. During therapy, all patients were observed closely for overt signs of toxicity. Temperature, pulse and respiration were noted every 4 hours during febrile periods. The diet during therapy consisted of foods to which the Miskito Indian had been long accustomed: rice, beans, cassava, beef, bread, and tropical fruits. The period of daily clinical observation whether in the hospital or on ambulatory therapy encompassed the entire 14 days required for treatment.

THERAPEUTIC RESULTS

Three different dosage schedules using primaquine were followed. To avoid any chance of an unfavorable reaction, the first treatment schedule used was based on a daily dose for adults of 10 mg. primaquine base and a total dose of 900 mg. chloroquine base, with younger people given proportionately less of each drug according to weight. Chloroquine was used in all groups because primaquine alone has a very slow and uncertain action against trophozoites. A special palatable syrup of primaquine was compounded for children so that fractional doses could be measured easily[5]. All patients who were found to be infected with malaria were treated as soon as they were found. A group of patients from one tropical village were set aside to serve as a therapeutic control and were treated with chloroquine alone. This group was selected several months after the other groups were started on treatment. A deliberate effort was made to treat all possible sources of infection in each locality. The combined effect of reducing the seed-bed by treatment and of reducing transmission by the use of residual DDT spray was expected to reduce the chance of reinfection to a very low level.

Group 1: Ten milligrams primaquine—A total of 121 patients received treat-

[5] Supplies of primaquine syrup and tablets were furnished gratis by Eli Lilly and Company, Indianapolis, Indiana, to whom thanks are extended. Primaquine is commercially available as a diphosphate salt, which contains 56.9 per cent primaquine base. Chloroquine diphosphate is commercially available under the trade name Aralen Diphosphate and contains 62 per cent chloroquine base.

ment according to a schedule based on a daily dose of 10 mg. primaquine base and a total dose of 900 mg. chloroquine base for an average adult (Table 6). All but one of these had infections caused by *P. vivax*. These patients were the first to receive primaquine therapy in this region and were treated in May, June and early July of 1951. There was no overt sign or symptom of toxicity in any individual in this group. Methemoglobin development ranged from 0.1 to 0.8 gm. per cent, the average being 0.19 gm. per cent. The white counts showed no significant change during treatment. Urinalyses revealed no abnormality. There were no allergic reactions or subjective complaints referrable to primaquine.

The follow-up of these patients at 60, 90, 120, 150 and 360 days showed only 4 cases of malaria, all of which proved to be *P. falciparum* and probably represented new infections.

TABLE 6

Proportional weight-dose schedule of primaquine and chloroquine for the group in which the average adult (127 lbs.) received 10 mg. primaquine base daily

WEIGHT (LBS.)	DRUGS*		NUMBER TREATED	AVERAGE CHANGE IN HEMOGLOBIN
	Primaquine (14 days)	Chloroquine (2 days)		
	(*mg. base/day*)			(*gm. per 100 cc*)
Up to 15	1.0	75	1	0.1
16 to 30	2.0	150	15	0.1
31 to 45	3.0	225	14	0.2
46 to 60	4.0	225	15	0.1
61 to 75	5.0	300	12	0.3
76 to 90	7.5	300	2	0.2
(Average adult 91 to 175)	10.0	450	62	0.3
Over 175	15.0	600	0	0

Total number treated, 121.

* Drug regimens in this table referred to as Schedule 1 in Table 9.

Group 2: Fifteen milligrams primaquine—A second group of 151 patients, all infected with *P. vivax*, received a dosage schedule based on a 15 mg. daily dose of primaquine base and a total dose of 900 mg. chloroquine base for an adult (Table 7). All of these were re-examined at 60, 90, 120, 150 and 360 days without evidence of malaria. This group was treated in the late summer of 1951. As with Group 1, there was no overt sign or symptom of toxicity during therapy. The methemoglobin produced during therapy averaged 0.23 gm. per cent with a range from 0.05 to 1.0 gm. per cent.

Group 3: Twenty milligrams of primaquine—A third group of 49 patients, all with *P. vivax* infections, received a dosage schedule based on a daily dose of 20 mg. primaquine base and a total dose of 900 mg. chloroquine base for adults (Table 8). This group was studied in the fall of 1951. None of these patients exhibited malarial parasites on subsequent examinations made 60, 90, 120, 150 and 360 days later. Even at this dose, no significant signs or symptoms of toxicity developed. Methemoglobin development ranged from 0.1 to 1.8 gm. per cent and

averaged 0.21 gm. per cent. The case showing maximum methemoglobin development had no clinical embarrassment from it.

Group 4 (controls): A small group (87 patients) drawn from the same area (Figure 1) as the therapeutic group were treated with a suppressive drug alone—

TABLE 7

Proportional weight-dose schedule of primaquine and chloroquine for the group in which the average adult (127 lbs.) received 15 mg. primaquine base daily

WEIGHT (LBS.)	DRUGS*		NUMBER TREATED	AVERAGE CHANGE IN HEMOGLOBIN
	Primaquine (14 days)	Chloroquine (2 days)		
	(mg. base/day)			*(gm. per 100 cc)*
Up to 15	1.5	75	3	0.6
16 to 30	3.0	150	31	0.1
31 to 45	4.5	225	18	0.2
46 to 60	6.0	225	16	0.1
61 to 75	7.5	300	9	0.2
76 to 90	10.0	300	10	0.2
(Average adult 91 to 175)	15.0	450	64	0.2
Over 175	21.5	600	0	0

Total number treated, 151.

* Drug regimens in this table referred to as Schedule 2 in Table 9.

TABLE 8

Proportional weight-dose schedule of primaquine and chloroquine for the group in which the average adult (127 lbs.) received 20 mg. primaquine base daily

WEIGHT (LBS.)	DRUGS*		NUMBER TREATED	AVERAGE CHANGE IN HEMOGLOBIN
	Primaquine (14 days)	Chloroquine (2 days)		
	(mg. base/day)			*(gm. per 100 cc)*
Up to 15	2.0	75	1	0
16 to 30	4.0	150	4	0.2
31 to 45	6.0	225	6	0.2
46 to 60	8.0	225	3	0.3
61 to 75	10.0	300	1	0.3
76 to 90	15.0	300	1	0
(Average adult 91 to 175)	20.0	450	32	0.5
Over 175	25.0	600	1	0.4

Total number treated, 49.

* Drug regimens in this table referred to as Schedule 3 in Table 9.

chloroquine, using the same dose based on weight as was used with the 3 primaquine-treated groups. This group was treated in the fall of 1951. As shown in Table 9, 6 relapses were found by examinations at 60, 90, 120, 150 and 360 day intervals.

No evidence of toxicity was demonstrated in any of the groups treated with

primaquine and chloroquine. Although hemolytic reactions have been reported by Hockwald *et al.* (1952), the doses of primaquine used in this study on natives of Eastern Nicaragua produced no severe hemolytic reactions.

TABLE 9

Therapeutic results of 4 treatment schedules using primaquine and chloroquine in combination and chloroquine alone in natives of Eastern Nicaragua suffering from infections of P. vivax

SCHEDULE NUMBER	DRUG SCHEDULE (ADULT DOSAGE)*		NUMBER TREATED	NUMBER RELAPSED (IN 1 YEAR)	PER CENT RELAPSED
	Primaquine (14 days)	Chloroquine (2 days)			
	(*mg. base/day*)				
1	10	450	121	0†	0
2	15	450	151	0	0
3	20	450	49	0	0
4	—	450	87	6	7.0
Treated with primaquine and chloroquine			321	0†	0
Treated with chloroquine only			87	6	7.0

* Individuals weighing less than 90 pounds were given proportionately smaller doses (see Tables 6, 7, 8).

† During the one year follow-up, 4 cases of *P. falciparum* were found in this group. These were re-infections.

INTERPRETATION OF RELAPSE PATTERN

It is apparent that chloroquine alone was followed by relatively few relapses and that chloroquine plus primaquine completely eliminated relapses. These results are consistent with either of two interpretations: (1) the residual tissue stages of endemic infections are few in number and are easily overcome by acquired immunity plus an effective suppressant, or (2) trophozoite infections may persist long after the tissue stages have become exhausted.

It has been shown elsewhere (Ruhe *et al.*, 1949; Alving *et al.*, 1953) that treatment late in the season of a bimodal form of *vivax* malaria is much more effective in reducing the relapse rate than is treatment early in the season. Because the control group treated with chloroquine was treated 6 months later than the initial group on primaquine, it is possible that this accounts for the relatively small number of relapses in the control group. It is likely that this malaria is bimodal in type because of the marked limitation of the transmission season by adverse rainfall during many months of the year.

In the 5 villages from which these groups were selected for treatment, a reexamination of 1,229 persons was made one year later. This represented nearly the entire population of the area. Of this group only 30 were found to be positive for malaria. This is a decline in the proportion of positive cases from 29.7 per cent to 2 per cent. Of these 30 positives, none had been treated during this program.

All individuals, whether treated with primaquine and chloroquine or with chloroquine alone, occupied dwellings sprayed with residual DDT during the

period of observation. The residual spraying and the intensive treatment, with virtual destruction of the local seed-bed of malaria, must have largely eliminated the chance of reinfections during the course of the year's observation.

The addition of the curative action of primaquine to the suppressive action of chloroquine in the treatment of this particular field infection of *vivax* malaria reduced the relapse rate from 7 per cent to zero (Table 9). To give a more precise evaluation of the curative action of primaquine it would be necessary to treat infections with a greater relapse potential.

After the main portion of this study had been completed, a survey was made of neighboring villages (Figure 1) which had received the same amount of residual DDT spray as had the villages from which the experimental groups were drawn. In all, 1,003 people were examined in May and August of 1952 of which 6.7 per cent were found positive for malaria.

DISCUSSION

Of importance in the field use of an antimalarial is the likelihood of serious toxicity, the frequency of minor toxicity (which will discredit a drug in the minds of the native population), and the possession of a "safety factor" type of toxicity. Almost all drugs, whether antimalarials or not, display serious reactions in certain especially sensitive people. It is now apparent that the incidence of very serious reactions to primaquine is extremely small, for in addition to the present series of 321 patients, a vastly larger group of American servicemen and prison volunteers have received equivalent or larger doses of the drug (Clayman *et al.*, 1952; Alving *et al.*, 1952). Because the average weight of the Nicaraguan adult was only 127 pounds, the daily dose for these people was proportionately higher per pound of body weight than is the same daily dose for the average American adult. The most serious reaction so far recorded has been a tendency to excessive hemolysis in about 5 per cent of American Negroes at daily doses of 30 mg. (base) or more (Hockwald *et al.*, 1952). This type of reaction was not observed in the present study.

Most drugs in common use have minor side effects which are more troublesome than dangerous. If these are frequent and distressing, a long-term, large-scale program of therapy will fail for lack of patient cooperation. Although primaquine does, in large doses or in occasional individuals, produce minor but distressing symptoms usually referrable to the gastrointestinal tract, this in no way interfered with the administration of the drug among the Nicaraguan natives who almost without exception eagerly solicited the drug. This occurred even though most of these patients had, in addition to chronic malaria, the usual intestinal parasites, anemia and poor nutrition. It is of interest that primaquine was easily tolerated by all age groups.

Lastly, a drug should produce premonitory and protective symptoms when a dangerously toxic dose is approached or an especially sensitive person is treated, especially if a drug is proposed for use on the broad unsupervised scale that is necessary for a practical antimalarial. Probably the most desirable protective toxic reaction for a drug designed for mass administration is gastrointestinal

upset and vomiting. This should occur at doses conveniently above the therapeutic level and comfortably below the lethal level. This type of "safety valve" toxicity improves rather than detracts from the value of a drug intended for mass therapy. In this particular respect primaquine is relatively well endowed, for at seriously high doses it produces vomiting and abdominal cramps (Clayman *et al.*, 1952) that will undoubtedly protect many from overdoses.

It remains then only to determine if the therapeutic activity of a drug is sufficiently great to meet the challenge of the prevailing strain of malaria. A drug for malaria must have an effective activity against the most refractory strains before it offers promise of general usefulness. Such has been the case with chloroquine as a suppressive agent, but there has not, until recently, been an active and safe curative agent for *vivax* malaria. The endemic malaria of Eastern Nicaragua has not presented a severe challenge to the curative action of primaquine. Controlled studies elsewhere against much more challenging strains (Southwest Pacific) have demonstrated the curative superiority of primaquine over all other curative agents (Edgcomb *et al.*, 1950). This difference highlights the great variation in malarias found in different parts of the world. It also serves to point out the fact that a curative agent is not always necessary in endemic areas in immune individuals. In this particular study, it is evident that the endemic malaria in highly immune individuals of Eastern Nicaragua was controlled best by chloroquine and primaquine given together, but the improved therapeutic action of these drugs in combination must be weighed in each case against the relapse potential of the prevailing strain of malaria and the increased cost and effort required for curative therapy as opposed to the simpler suppressive therapy.

CONCLUSION

Primaquine was found to be therapeutically effective in 321 chronic cases of naturally acquired *P. vivax* in Eastern Nicaragua at doses as low as 10 mg. base daily for 14 days for an average adult. Furthermore it proved to be safe and well tolerated by persons of all ages when given in doses of twice this amount. Treatment schedules for all ages based on weight are presented. It is felt, however, that the malaria found in Eastern Nicaragua does not present a severe therapeutic challenge.

It is of interest that after the use of DDT residual spray for 9 months there was still a 29.7 per cent incidence of malaria. The combination of DDT and intensive curative therapy for the next 15 months reduced the incidence to 2 per cent of the total population. At the same time a group of 1,003 people in neighboring villages which had received the same residual DDT spray program without antimalarial therapy presented a malaria incidence of 6.7 per cent.

REFERENCES

ALVING, ALF S., ARNOLD, J., AND ROBINSON, D. H., 1952. Status of primaquine: 1. Mass therapy of subclinical vivax malaria with primaquine, *J.A.M.A.* **149:** 1558–1570.

ALVING, ALF S., HANKEY, D. D., COATNEY, G. R., JONES, R. JR., COKER, W. G., GARRISON, P. L., AND DONOVAN, W. N., 1953. Korean vivax malaria: II. Curative treatment with pamaquine and primaquine. In press.

CLAYMAN, C. B., ARNOLD, J., HOCKWALD, R. S., YOUNT, E. H., JR., EDGCOMB, J. H., AND ALVING, ALF S., 1952. Status of primaquine: 3. Toxicity of primaquine in Caucasians, *J.A.M.A.* **149:** 1558–1570.

EDGCOMB, J. H., ARNOLD, J., YOUNT, E. H. JR., ALVING, ALF S., JEFFERY, G. M., EYLES, D., AND YOUNG, M. D., 1950. Primaquine, SN 13272, a new curative agent in vivax malaria: A preliminary report, *J. Nat. Malaria Soc.* **21:** 285–292.

HOCKWALD, R. S., ARNOLD, J., CLAYMAN, C. B., AND ALVING, ALF S., 1952. Status of primaquine: 4. Toxicity of primaquine in Negroes, *J.A.M.A.* **149:** 1558–1570.

RUHE, D. S., COOPER, W. C., COATNEY, G. R., AND JOSEPHSON, E. S., 1949. Studies in human malaria: XV. The therapeutic action of pamaquine (Plasmochin) against St. Elizabeth strain vivax malaria, *Amer. Jour. Hyg.* **49:** 367–373.

A FURTHER STUDY OF THE TOXICITY OF PYRIMETHAMINE (DARAPRIM) IN MAN[1]

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The toxicity of pyrimethamine (Daraprim), 2,4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine, an antimalarial agent, was recently discussed by Myatt *et al.*, (1953). In that study 12 white male subjects were given 25 mg. of the drug in a single dose daily for a period of 7 weeks, far in excess of what is needed for therapy or suppression. Toxicity, evidenced by a megaloblastic type of anemia, occurred in six individuals.

The initial study was extended, as reported here, in order to test for toxicity at a dosage sufficient for suppression, and to determine whether white and Negro subjects are equally tolerant to such dosage.

MATERIALS AND METHODS

Thirty-five volunteers, 25 Negroes and 10 whites, from the inmate population of the U. S. Penitentiary, Atlanta, Georgia were included in the study. These men were from 23 to 43 years of age and varied in weight from 105 to 272 pounds. All subjects were in excellent health as shown by histories, physical examinations and laboratory tests.

Each volunteer was given a single 25-mg. dose of pyrimethamine weekly, starting 27 January 1953 and continuing on the same day each week through 21 July 1953. Due to institutional transfers one Negro volunteer received only 8 doses and another only 19 doses of the drug. The remaining 33 subjects received in all 26 doses during the 6-month period. Histories were taken and physical examinations were done prior to starting the drug, and at weekly intervals during the drug administration. In addition, the following laboratory procedures were performed on each man: red blood cell count, hemoglobin determination, hematocrit, total white blood cell count, differential white blood cell count, and urinalysis. Sternal marrow aspirations were done on 34 subjects at the end of 3 months and on 33 subjects at the end of 6 months.

RESULTS

During the drug administration period no symptoms were reported by any of the volunteers and no abnormal physical findings were found. Red blood cell counts, hemoglobin determinations, hematocrits, total white blood cell counts, differential white blood cell counts, and urinalyses all remained within normal limits. The sternal marrow studied at 3 and 6 months was normal.

[1] The work reported here was conducted under a contract between the Office of the Surgeon General, Department of the Army, and the National Institutes of Health.

SUMMARY AND CONCLUSIONS

Reported therapeutic tests show that pyrimethamine is an effective suppressant for malaria in dosage of 25 mg. single dose weekly. Our studies reported here and previously (Coatney *et al.*, 1953) indicate that this dosage may be given safely to white or colored races without untoward reactions, barring possible cases of idiosyncrasy.

ACKNOWLEDGMENTS

We are indebted to our consultant hematologist, Dr. Charles M. Huguley, Jr., Assistant Professor of Medicine, Emory University School of Medicine, who examined and interpreted all the sternal marrow smears. We are also indebted to all those individuals whose help was acknowledged in our preceding papers on pyrimethamine.

REFERENCES

Coatney, G. R., Myatt, A. V., Hernandez, T., Jeffery, G. M., and Cooper, W. C., 1953. Studies in human malaria. XXXII. The protective and therapeutic effects of pyrimethamine (Daraprim) against Chesson strain *vivax* malaria, *Am. J. Trop. Med. & Hyg.* **2**: 777–787.

Myatt, A. V., Hernandez, T., Coatney, G. R., 1953. Studies in human malaria. XXXIII. The toxicity of pyrimethamine (Daraprim) in man, *Am. J. Trop. Med. & Hyg.* **2**: 788–795.

SOME INTERACTIONS *IN VITRO* OF *ENDAMOEBA HISTOLYTICA* AND SINGLE SPECIES OF MICROBIAL SYMBIONTS

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For a number of years the dominant aim of investigators of the physiological reactions of *Endamoeba histolytica* has been to obtain pure cultures of the parasite in order that its metabolism may be studied without complications attributable to the presence of other microorganisms. As was pointed out in our earlier publications (Rees *et al.*, 1941, 1941a) this problem is more difficult than those on the cultivation of several other parasites that live in the host with bacteria. Many attempts, not requiring specific citation here, to obtain growth of *E. histolytica* from bacteria-free cysts or trophozoites have not been successful. However, the recent work of Shaffer and Sienkiewicz (1952) in which the ameba was grown without other microorganisms in minced chick embryo constitutes a notable advance toward the goal of cultivating the ameba free from all other living cells. We are pleased to report that our efforts to cultivate the ameba by their method have been successful.

In 1942 our microisolation technique (Rees, 1942) provided a method of establishing cultures of *E. histolytica* with any single species of microorganism with which the ameba will grow. Since that time a number of antibiotics have been made available as bacteriostatic agents. Antibiotics have been used by Jacobs (1947) in eliminating *Clostridium perfringens* from cultures of the ameba with this bacterium and in obtaining limited growth of the ameba with heat-killed bacteria. Although more time-consuming than the microisolation technique, the antibiotic approach has been found useful by us and by other investigators. In addition, with leads furnished by the investigations of Shaffer and Frye (1948) it has been possible to cultivate the ameba with *Trypanosoma cruzi* (Phillips, 1950; Phillips and Rees, 1950).

Up to the present time the cultures of *E. histolytica* with single species of microbial symbionts have had limited usefulness in studies on the metabolism of the ameba since, with several exceptions (Snyder *et al.*, 1943; Rees *et al.*, 1944), the only detectable actions have been those of the symbiont. In fact, the word "symbiont" used in an early paper (Rees *et al.*, 1942) was replaced in later work with "associate" (Reardon *et al.*, 1952) because it appeared that the ameba contributed nothing toward growth of the companion microorganism. However, experiments herein reported indicate symbiosis between the ameba and selected single species of bacteria. In view of the occurrence of bacteria with amebae in the host it is axiomatic that methods of measuring interactions between these organisms are prerequisite to an understanding of the role of *E. histolytica* as an infective and pathogenic agent in intestinal amebiasis.

[1] Laboratory of Tropical Diseases.

MATERIALS AND METHODS

Some of the methods were employed in work reported before the American Society of Tropical Medicine in 1951 (Rees, 1951)[2] and other methods in experiments conducted since that time. To date, 17 ameba-bacterial cultures have been studied as indicated in Figure 1. Some of the species of bacteria were available in our Laboratory, some were supplied by the Laboratories of Biologics Control, Infectious Diseases and Physical Biology also of the National Institutes of Health. Some experiments were also conducted with cultures of *E. histolytica-*

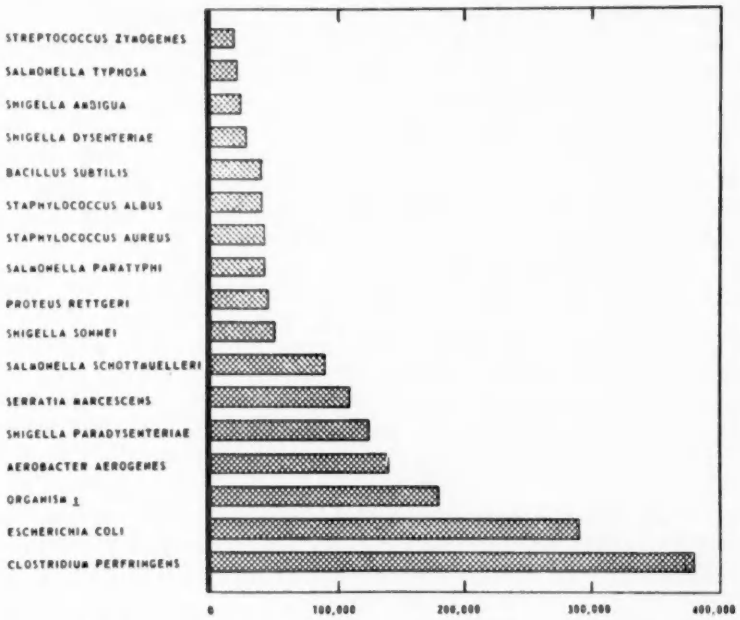

FIG. 1. Comparative yields of *Endamoeba histolytica* from test tube cultures with indicated single species of bacteria in whole-egg medium. Numbers of amebae indicated on the abscissa.

T. cruzi from which source the cultures of Figure 1 with designated bacterial symbionts were obtained. Except as otherwise indicated, whole-egg medium was used for the ameba cultures as well as for bacteria cultures. The whole-egg base was overlaid with Stone's Locke solution (Stone, 1935) enriched with rice flour[3] but not with serum, and unless otherwise stated, not with sugar. Well standard-

[2] Only the title of this report was published.

[3] The rice flour was ground and sifted through bolting cloth of the finest mesh by the Grain Branch Section, Production and Marketing Administration, U.S.D.A., from unpolished rice obtained through the generosity of the Louisiana State Rice Milling Company.

ized methods employed by the Media Preparation Section of NIH provided medium in which, excepting the rice flour, the proportions of ingredients were constant. To 270 cc. of whole egg emulsion, 75 cc. of sugar-free Stone's Locke's solution was added, thoroughly mixed with the emulsion, and the mixture subjected to a partial vacuum to remove air bubbles. This material was then dispensed in 16 x 150 mm. test tubes in the amount of 3.5 cc. per tube. Uniform slants were then prepared by inspissation in the autoclave, and to each slant 5 cc. of sugar-free Locke's solution was then added. The medium was sterilized by autoclaving and after cooling, the preparation of the medium was completed by adding rice flour in the amount of approximately 20 mg. per tube. Variations in rice flour content were due to difficulties of accurate measurement without bacterial contamination. Analysis of glucose that diffused from the egg base into the overlay showed that after 96 hours at 37° C. the sterile overlay contained on the average 0.04 per cent glucose.

Medium prepared from egg white instead of whole-egg was used in designated cases, as well as whole-egg medium with an overlay of 12½ per cent gelatin. Unless otherwise noted the amebae were propagated from seventy-two hour cultures with an inoculum of 0.5 cc. obtained from the sediment of the culture. The numbers of amebae that grew in each ameba-bacterial culture were calculated from counts in the Neubauer hemocytometer, the amebae being counted from five of the millimeter squares in each chamber. Each experimental run comprised five tubes from each of which two complete counts requiring four random samples were made. The samples were obtained by pipetting the bottom 3 cc. of the overlay to a 100 cc. beaker, shaking the latter, inserting a Pasteur pipette, and transferring liquid that freely entered the capillary end to the hemocytometer. A statistical analysis of this method[4] (unpublished) shows that on the average the coefficient of variation was 17 per cent. The control culture of the bacterium alone was propagated in whole-egg medium with rice flour from inocula of the same volume as for the ameba culture. The growth of the bacterium was estimated from turbidimetric readings in the Beckman spectrophotometer. These readings were obtained from the entire overlay by transferring it to Wasserman tubes and spinning for five minutes, not counting stopping time, in a #590 Sorvall angle head centrifuge at 1,500 r.p.m. The centrifugal force was calculated[5] at 287 × G. Adoption of this time and speed of spinning was based on a number of trial runs at different times and speeds of spinning to ascertain when particles larger than bacterial cells were sedimented. For comparable readings on ameba-bacterial cultures, bacterial cultures, and sterile medium, five tubes from each source were spun simultaneously. Turbidity was measured in a 1 cm. cell and expressed as log I_0/I at 520 millimicrons.

The figures obtained for carbon dioxide production from cultures of the amebae with a bacterium, and of the bacterium by itself, were divided by the respective turbidity to ascertain whether increased production of the gas was due to in-

[4] These calculations were made through the kindness of Mr. Nathan Mantel of the National Cancer Institute.

[5] Calculation made by Ivan Sorvall, Inc.

creased growth of the bacterium. However, errors which were attributable to nonsedimented material in the ameba cultures were introduced into these calculations. These errors were of significance only when small quantities of carbon dioxide were produced.

Comparative amounts of carbon dioxide produced in cultures of ameba-bacteria, and of bacteria, were measured by the Eldredge-tube method (Werkman, 1946) using a modified tube as illustrated in Figure 3. The organisms were grown in the "test tube" arm and standardized $Ba(OH)_2$ was dispensed in the "boat." The stock $Ba(OH)_2$ was titrated against N/10 potassium acid phthalate using phenolphthalein as indicator. At indicated periods of incubation a 10 cc. aliquot of the alkali was titrated against the acid and from differences between the two titrations the amount of carbon dioxide absorbed was calculated in cc. In his discussion of the Eldredge-tube, Dr. Werkman (1946) suggested than an incubation period of two weeks may be needed for absorption of all of the carbon dioxide produced by a culture of bacteria. In the present investigation, however, the objective was to ascertain differences between amounts of the gas produced from two kinds of cultures under uniform conditions of inoculation and uniform times of incubation. The titrations were made at periods of incubation from 24 to 96 hours. Trial runs on standardized solutions of sodium bicarbonate, acidulated through a stop cock with sulfuric acid showed recoveries averaging 85 per cent after 96 hours at 37°C. Determinations of pH were made in the Beckman pH meter.

EXPERIMENTAL DATA

The data on cultures of *E. histolytica* with bacterial symbionts are illustrated in Figures 1 and 2. Although Nakamura (1952) has reported failure to establish *E. histolytica* in a liver-protease peptone medium with the "streptobacillus" of Shaffer and Frye (1948), or with organism *t* from an inoculum of *E. histolytica-T. cruzi*, Luttermoser and Phillips (1952) succeeded with transfers to whole-egg medium in establishing ameba cultures with organism *t*. However, in many cases amebae failed to grow from such inocula. In our investigations an inoculum of 1 cc. from well-mixed ameba-trypanosome cultures was required to establish the amebae in medium previously seeded with symbionts listed in Figure 1. In all cases the trypanosome failed to grow in whole-egg medium and disappeared at the second or third serial transfer. The ameba cultures showed striking differences among the species of bacteria with regard to the numbers produced. For example, *Streptococcus zymogenes*, *Salmonella typhosa*, and two species of *Shigella* produced respectively about one-tenth as many amebae as *Clostridium perfringens*. The factors involved in these differences are unknown but their investigation is a challenging problem. Some data on utilization by the ameba of ingredients of the medium are available. The parasite ingests starch grains (Dobell *et al.*, 1926) and will not grow without cholesterol (Snyder *et al.*, 1943; Rees *et al.*, 1944). Our data on the requirement of cholesterol by *E. histolytica* (Rees *et al.*, 1944) were obtained from cultures with organism *t* in egg white medium (Rees *et al.*, 1945, 1945a, 1947) using inocula from cultures of whole-egg medium.

The experiments showed that enrichment of the egg white medium with B vitamins and cholesterol was required for growth of the ameba, the vitamins but not cholesterol being required by organism *t*. However, as illustrated in Figure 2, the vitamin-cholesterol enrichment was inadequate for indefinite maintenance of the amebae through serial transfers. On the other hand, Figure 2 shows that maintenance of *E. histolytica-Escherichia coli* has occurred in egg white medium with cholesterol, without vitamin enrichment. The series furnishing material for Figure 2 was started in January, 1952, and is still in cultivation. The requirement for B vitamins by *E. histolytica*-organism *t* in contrast with *E. histolytica-E. coli* is not surprising since, as stated by Gale (1951), *E. coli* produces B vitamins. The continued maintenance of one ameba-symbiont

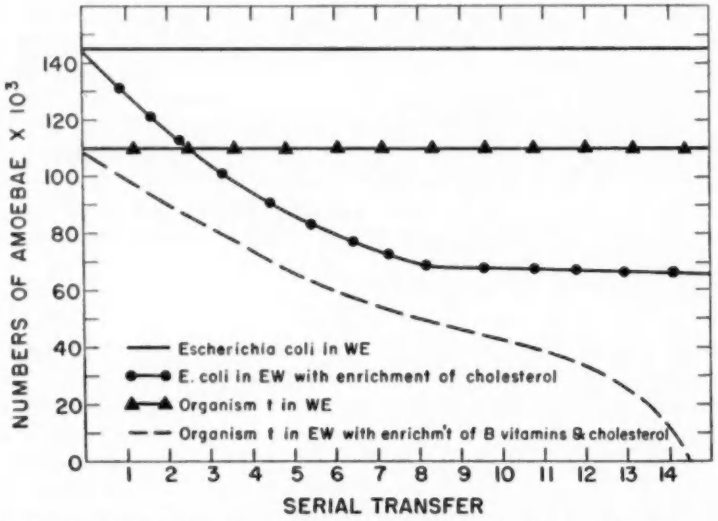

FIG. 2. Growth of *Endamoeba histolytica* with *Escherichia coli* in whole-egg (WE) and egg white (EW) medium, and of *E. histolytica*-organism *t* in the respective media.

combination and failure of maintenance of the other are attributable to unknown differences in metabolism of the bacteria. The failure of the cultures of *E. histolytica-E. coli* to obtain the level of ameba growth in egg white medium that has been found in whole-egg medium likewise requires explanation. For several months, a series of *E. histolytica-A. aerogenes* was maintained in egg white medium with cholesterol enrichment.

With reference to enzymes of *E. histolytica*, our data indicate production of gelatinase. Interest in gelatinase was aroused by a report of Dr. Verder, unpublished, showing that organism *t* has no gelatinase. The following experiment is typical of many that have been performed: two tubes of the gelatin medium were inoculated from a 72-hour culture of *E. histolytica*-organism *t*, two tubes with organism *t* only, and two with sterile Locke's solution. After incubation for four

days at 37°C. the tubes were removed to the refrigerator at 10°C. When examined three hours later the overlay of the ameba tubes was still liquid while that of the tubes with organism *t*, and of the sterile tubes, was completely gelled. The possible function of this protease in the metabolism of *E. histolytica* is unknown but in the light of theories that this ameba produces a protease or some substance that may lyse the cells of the host, further work is needed with the objective of clarifying the role of the gelatinase.

Questions of primary importance are whether the liberation of starch grains in cultures of *E. histolytica* with single species of bacteria, as previously reported for *E. histolytica*-organism *t* (Reardon *et al.*, 1949), is effected by a proteolytic enzyme, whether the enzyme is produced by the ameba, and whether such production may be adaptive or constitutive as defined by Gale (1951). Observations on all of the cultures of Figure 1 have shown that without exception the starch grains were liberated. Starch grain liberation by *E. histolytica*-organism *t* is illustrated in Figure 4 which indicates also the absence of such liberation by organism *t* alone. According to Rehwald (1926) the starch of cereals is intimately associated with protein substances. He states also that in the manufacture of rice starch from ground rice advantage is taken of the solubility of the protein matrix in dilute solutions of caustic soda. To demonstrate liberation of starch grains from our preparation of ground rice, several cc. of sodium carbonate solution at pH 9.5 were added to approximately 1 gm. of the rice flour in a test tube and the preparation held at 37°C. After a period of 24 hours the sediment of the tube showed numerous starch grains indistinguishable from those observed in ameba cultures. To illustrate fragmentation by proteolytic enzymes, filtrates of protease solutions were added to sterile medium. When added in 1 cc. amounts per tube of whole-egg medium, bacteria-free solutions of commercial papain, ficin, and crystallized trypsin (Armour & Co.) respectively, liberated the starch after one to four days at 37°C., the microscopic picture being indistinguishable from that produced in the ameba cultures. With respect to the 17 species of bacteria listed in Figure 1, only *Bacillus subtilis* has produced starch-grain liberation in pure culture. Fragmentation of the rice was produced by bacteria-free filtrates of cultures of *B. subtilis* either with or without amebae, but thus far all attempts to obtain this result from filtrates of cultures of *E. histolytica* with other single species of bacteria have failed, probably because the enzyme acts only in small loci of effective concentration.

It was of interest, therefore, to ascertain whether rice proteolysis could be demonstrated in cultures of *E. histolytica-T. cruzi*. Accordingly some of these cultures were enriched with rice flour and sediment from them was examined for evidence of rice proteolysis for periods up to 10 days of incubation. Starch grains were not liberated although occasional amebae were seen with ingested particles of rice. When, on the other hand, amebae from the cultures with *T. cruzi* were inoculated into whole-egg medium previously seeded with organism *t*, the liberation of starch grains was noted after overnight incubation. When rice was added to cultures of *E. histolytica*-"streptobacillus" in the medium of Shaffer and Frye (1948) there was evidence of scanty starch-grain liberation detectable only

at periods of incubation of five days or longer. The data lend some support to a theory that starch-grain liberation by *E. histolytica* is adaptive. It is possible, however, that symbiosis between the ameba and species of bacteria is necessary.

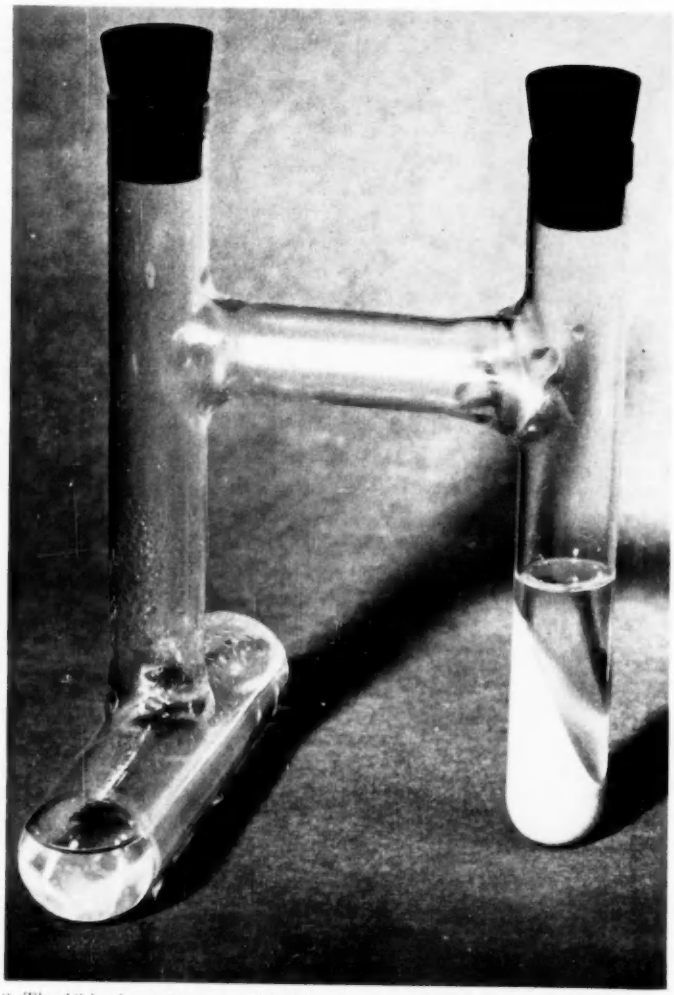

FIG. 3. The Eldredge-tube as modified for measurement of carbon dioxide from cultures of *Endamoeba histolytica*.

Accumulation of a glycogen mass in the amebae was observed regardless of whether they were grown with a bacterium in medium enriched with rice, or without rice but with glucose in the medium of Phillips (1950) with *T. cruzi*, or that of Shaffer and Frye (1948) with the "streptobacillus." Glycogen was

demonstrated by the Bauer technic as specified by Lillie (1948). The accumulation of glycogen within the ameba indicates that it transforms the rice starch into glycogen, and that in culture with *T. cruzi* it may utilize glucose for glycogen synthesis. Experiments with the objective of clarifying these reactions are in progress.

The amounts of carbon dioxide produced from Eldredge tubes containing *E. histolytica* with *A. aerogenes*, organism *t* and *C. perfringens*, respectively, are shown in Tables 1, 2 and 3. With the first two species of bacteria the amebae produced significantly more carbon dioxide than was produced by the bacteria when grown without the amebae except in the cultures with *A. aerogenes* that were enriched with one per cent glucose. As has already been stated, the glucose content of the glucose-unenriched sterile medium was scanty, and as shown by

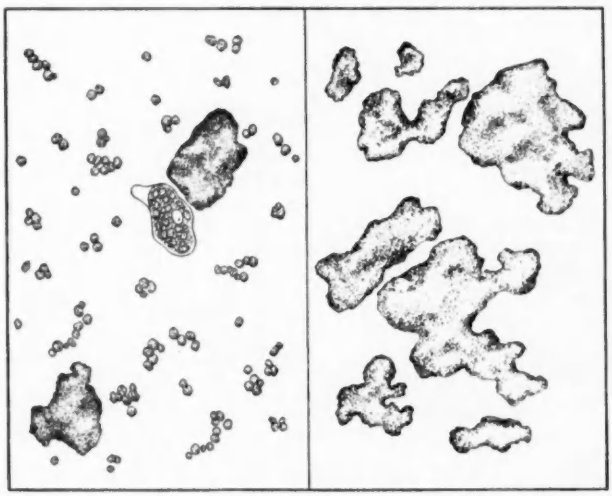

FIG. 4. Effects produced in rice flour in cultures of *Endamoeba histolytica*-organism *t* and of organism *t* without ameba (right).

von Brand *et al.* (1943) the glucose was utilized almost to the vanishing point during the first 24 hours of incubation of *E. histolytica*-organism *t*, or of organism *t*. These data have been confirmed by determinations made during the present investigation, and extended to include the cultures of ameba-bacteria and of bacteria of Tables 1, 3 and 4. In cultures that were not enriched with glucose there was richer growth of *A. aerogenes* and organims *t* respectively in the presence of amebae than in their absence, as indicated by higher turbidities. The ratios CO_2/T were also higher, showing that increased substrate was available for the bacteria. The magnitude of the ratio was dependent on the level of substrate available as shown by the data of Table 1 from glucose-enriched cultures. The significance of the increased ratio CO_2/T in the ameba-bacterium cultures over the bacterium cultures may be explained by extra substrate furnished by the amebae acting on rice flour, first with a protease to liberate starch

grains, and then with amylase to liberate glucose or maltose. Further work on this hypothesis is in progress.

In sharp contrast with the results summarized in Tables 1 and 2, the figures of Table 3 show no differences between amounts of carbon dioxide produced from *E. histolytica-Clostridium perfringens* and from *C. perfringens*. However, the bacterium without amebae produced almost a three-fold increase of the gas

TABLE 1

Comparative determinations of carbon dioxide and turbidity from Eldredge-tube cultures of Endamoeba histolytica-Aerobacter aerogenes *and* A. aerogenes *at 96 hours of incubation*

FIVE-TUBE SET OF CULTURES	AMEBAE PER TUBE × 10^3	*E. h.-A. aerogenes*			*A. aerogenes*		
		CO_2 in cc.	Turbidity Log I_0/I	CO_2/T	CO_2 in cc.	Turbidity Log I_0/I	CO_2/T
1	57.5	3.69	0.515	7.2	2.13	0.396	5.4
2	56.0	4.72	0.552	8.5	1.90	0.325	5.8
3	52.0	4.70	0.573	8.2	1.85	0.382	4.9
4	52.0	4.87	0.537	9.1	1.85	0.390	4.7
Average	54.4	4.49	0.544	8.3	1.94	0.374	5.2
5*	—	14.78	1.210	12.2	16.32	1.010	16.1

* Enriched with glucose in the amount of one per cent.

The figures on carbon dioxide were corrected for changes in titer of the barium hydroxide attributable to absorption of carbon dioxide from the air as ascertained from titrations on sterile medium.

TABLE 2

Comparative determinations of carbon dioxide and turbidity from Eldredge-tube cultures of Endamoeba histolytica-*organism* t *and organism* t

FIVE-TUBE SET OF CULTURES	HOURS OF INCUBATION	AMEBAE PER TUBE × 10^3	*E.h.*-ORGANISM *t*			ORGANISM *t*		
			CO_2 in cc.	Turbidity Log I_0/I	CO_2/T	CO_2 in cc.	Turbidity Log I_0/I	CO_2/T
1	96	123.3	3.86	0.449	8.6	2.30	0.351	7.0
2	72	182.7	3.08	0.375	8.2	2.41	0.304	8.0
3	72	116.4	3.12	0.377	8.3	1.46	0.333	4.4
4	96	110.7	4.03	0.413	9.7	2.02	0.277	7.3
Averages		133.4	3.52	0.403	8.7	2.05	0.316	6.6

over amounts produced by *A. aerogenes* or organism *t*. It was of interest, therefore, to ascertain whether *C. perfringens* might utilize rice flour, and for this objective the experiments summarized in Table 4 were conducted, in which four species of bacteria were cultivated without amebae in media that were enriched, or not enriched with rice flour. The results show a four-fold increase in the amount of carbon dioxide produced by *C. perfringens* from the medium with rice over the rice-free medium. The turbidity determinations and the ratios

CO_2/T show that the increased gas production was due to greater activity of the bacteria present rather than to the actual increase in numbers of bacteria. Utilization of the rice may, therefore, explain the lack of dependence of *C. perfringens* or substrate resulting from the metabolism of the amebae. The data on the three

TABLE 3

Comparative determinations of carbon dioxide and turbidity from Eldredge-tube cultures of Endamoeba histolytica-Clostridium perfringens *and* C. perfringens

FIVE-TUBE SET OF CULTURES	HOURS OF INCUBATION	AMEBAE PER TUBE × 10^3	*E.h.-C. perfringens*			*C. perfringens*		
			CO_2 in cc.	Turbidity Log I_0/I	CO_2/T	CO_2 in cc.	Turbidity Log I_0/I	CO_2/T
1	72	138.3	7.00	0.493	14.0	7.22	0.506	14.2
2	96	27.3*	7.72	0.441	17.5	7.60	0.519	14.6
3	96	12.0	9.05	0.509	15.1	9.12	0.495	18.4
Averages		—	7.92	0.481	15.5	7.98	0.506	15.7

* Amebae reach maximum growth with *C. perfringens* at 48 hours of incubation.

TABLE 4

Comparative determinations of carbon dioxide from indicated species of bacteria at 72 hours of incubation in medium with and without rice flour

BACTERIUM	FIVE-TUBE SET OF CULTURES	R-PLUS			R-MINUS		
		CO_2 in cc.	Turbidity Log I_0/I	CO_2/T	CO_2 in cc.	Turbidity Log I_0/I	CO_2/T
Aerobacter aerogenes	1	2.97	0.679	4.4	1.85	0.499	3.7
	2	1.96	0.482	4.0	1.61	0.480	3.4
Averages		2.46	0.580	4.2	1.73	0.489	3.5
Escherichia coli	1	1.96	0.541	3.6	1.23	0.596	2.2
	2	2.02	0.493	4.1	1.85	0.409	4.5
Averages		1.99	0.517	3.8	1.54	0.489	3.4
Organism *t*	1	2.41	0.223	10.8	1.56	0.502	9.0
	2	3.19	0.493	8.1	1.36	0.163	8.3
Averages		2.80	0.358	9.4	1.46	0.166	8.7
Clostridium perfringens	1	7.11	0.561	13.0	1.74	0.449	3.9

species of other bacteria indicate some utilization of the rice which may be explainable in part to traces of glucose in the rice flour. Reducing substances, principally glucose, may be extracted from the flour by water to the extent of 1.5 μg. per mg. of the flour.

According to Bradin and Kun (1952) there was evidence of production of

hydrogen sulfide from washed suspensions of *E. histolytica* with added cysteine. Therefore, in view of the occurrence of cysteine in egg protein, it was of interest to ascertain whether sufficient hydrogen sulfide might have been produced in our cultures to affect the titrations of the barium hydroxide. Moistened strips of lead acetate paper suspended in the tubes during incubation showed no blackening. It appears, therefore, that our data were not invalidated by minute amounts of this gas that might have been produced.

Since the pH at which microorganisms are grown is known to have an effect on amounts of carbon dioxide that may be liberated, a large number of determinations of pH were made on sterile medium and on cultures of the organisms, furnishing data for Tables 1 to 3. The average reading for sterile medium was 7.6. For cultures of *E. histolytica-A. aerogenes*, and of *A. aerogenes*, the pH was 7.2 at 24 hours of incubation, with no changes thereafter and no differences between cultures with and without amebae. The average pH of cultures of *E. histolytica*-organism *t* and of organism *t*, was 6.2 and 6.4 respectively, excepting in an experiment not reported in Table 2 where glucose was added when the pH dropped to 5.2. The pH of cultures of *E. histolytica-C. perfringens* and of *C. perfringens* dropped from 6.1 at 24 hours to 5.9 at 96 hours. It does not appear that the slight changes of pH, when such occurred, between the cultures with and without amebae were sufficient to affect the validity of the data reported on carbon dioxide. Comparative titrations of sterile medium acidified with sulfuric acid and not acidified, at 96 hours of incubation, showed only slight differences beyond the experimental error. These results show that amounts of alkali reserve in our medium were insufficient to invalidate the results.

DISCUSSION

The data of the present report show unequivocally that more carbon dioxide was produced in cultures of *E. histolytica* with *Aerobacter aerogenes* and with organism *t* than in cultures of the respective species of bacteria without amebae. The data furnish strong indications that increased production of the gas resulted from richer growth and greater activity of the bacteria. The question whether some carbon dioxide was produced by the ameba *per se* has not been answered. Fragmentation of the rice by a protease of the ameba and hydrolysis of starch by amylase produced substrate for growth of the bacteria. Increased production of carbon dioxide attributable to the presence of the amebae was demonstrable when the glucose content of the medium was scanty and when the bacterial symbiont did not utilize the rice. On the other hand when the amebae were grown with *Clostridium perfringens* (which when grown by itself produced approximately four times as much carbon dioxide in medium enriched with rice as in medium without rice) no increase in the gas attributable to the amebae could be demonstrated. Such increase was likewise not demonstrable when the medium contained considerable amounts of glucose such as that used for *E. histolytica*-"streptobacillus" or *E. histolytica-T. cruzi*.

Although the Eldredge-tube method of approach to problems on the carbohydrate metabolism of *E. histolytica* has obvious limitations, its value for meas-

urement of an end product is herein demonstrated. The spectrophotometric method of measuring turbidities as an indication of bacterial growth, as herein modified, also has limitations due to some cloudiness in the ameba cultures. However, the readings on turbidity show fairly good correlation with measurements of carbon dioxide. As indicated early in the present report there is need for pure cultures of *E. histolytica* for investigations on its metabolism and for other studies. However the present report shows that information is obtainable from cultures of the ameba with single species of companion microorganisms.

SUMMARY

Determinations on the numbers of amebae obtainable from cultures of *E. histolytica* with 17 individual species of bacterial symbionts in whole-egg medium showed differences ranging from 20,000 to 450,000 amebae per culture.

E. histolytica-organism *t* could not be propagated through indefinite serial transfer in egg white medium even with enrichments of cholesterol and B vitamins. With enrichment of cholesterol the ameba was propagated in egg white medium with *Escherichia coli* and *Aerobacter aerogenes*, respectively, through indefinite serial transfer.

Evidence was obtained indicating that *E. histolytica* produces a gelatinase and/or a protease that liberates starch grains from particles of ground rice, and that rice proteolysis may be adaptive.

Using the Eldredge-tube method of measuring carbon dioxide, more of the gas was demonstrable from cultures of *E. histolytica* with two bacterial symbionts than from the symbiont alone except when glucose-enriched medium was used. The increase was attributable to substrate furnished through the metabolism of the amebae. No differences in production of carbon dioxide were demonstrable from cultures of *E. histolytica-Clostridium perfringens*, and of *C. perfringens* without amebae. This result was attributed to utilization of rice flour by *C. perfringens*.

REFERENCES

BRADIN, J. L., JR., AND KUN, E., 1952. Studies on the metabolism of *Endamoeba histolytica*, *J. Parasitol.* **38:** Sec. 2, 13.

VON BRAND, T., REES, C. W., JACOBS, L., AND REARDON, L. V., 1943. Studies on reducing substances and gas formation in cultures of *Endamoeba histolytica* and a single species of symbiotic bacteria, *Am. J. Hyg.* **37:** 310–319.

DOBELL, C., AND LAIDLAW, P. P., 1926. On the cultivation of *Entamoeba histolytica* and some other entozoic amoebae, *Parasitol.* **18:** 283–318.

GALE, E. F., 1951. *The Chemical Activities of Bacteria*, Academic Press Inc., New York, 213 pp.

JACOBS, L., 1947. The elimination of viable bacteria from cultures of *Endamoeba histolytica* and the subsequent maintenance of such cultures, *Am. J. Hyg.* **46:** 172–176.

LILLIE, R. D., 1948. *Histopathological Technic*, The Blakiston Company, Philadelphia, 300 pp.

LUTTERMOSER, G. W., AND PHILLIPS, B. P., 1952. Some effects of cultural associates on the infectivity of a strain of *Endamoeba histolytica* for the rabbit, *Am. J. Trop. Med. & Hyg.* **1:** 731–735.

NAKAMURA, M., 1952. Attempts to exchange associated organisms in cultures of *Endamoeba histolytica*, *J. Parasitol.* **38:** 83.

PHILLIPS, B. P., 1950. Cultivation of *Endamoeba histolytica* with *Trypanosoma cruzi*, *Science* **111**: 8–9.

PHILLIPS, B. P., AND REES, C. W., 1950. The growth of *Endamoeba histolytica* with live and heat-treated *Trypanosoma cruzi*, *Am. J. Trop. Med.* **30**: 185–191.

REARDON, L. V., AND BARTGIS, I. L., 1949. The cultivation of the small race of *Endamoeba histolytica* with a single species of bacteria, *J. Parasitol.* **35**: 218.

REARDON, L. V., VERDER, E., AND REES, C. W., 1952. The cultural requirements of *Endamoeba coli* and the comparative effects of drying on the cysts of *E. coli* and *E. histolytica*, *Am. J. Tropl Med. & Hyg.* **1**: 155–161.

REES, C. W., 1942. The construction of a micromanipulator for the isolation of protozoa, *Am. J. Trop. Med.* **22**: 487–492.

REES, C. W., 1951. Some experiments on the utilization of carbohydrates by *Endamoeba histolytica*, Title only of presentation before 47th Ann. Meet. Am. Soc. Trop. Med., Chicago, 1951.

REES, C. W., BOZICEVICH, J., REARDON, L. V., AND DAFT, F. S., 1944. The influence of cholesterol and certain vitamins on the growth of *Endamoeba histolytica* with a single species of bacteria. *Am. J. Trop. Med.* **24**: 189–193.

REES, C. W., BOZICEVICH, J., REARDON, L. V., AND JONES, F., 1942. A preliminary note on the complement fixation test for amoebiasis with antigens prepared from *Endamoeba histolytica* grown with a single species of bacteria. *Am. J. Trop. Med.* **22**: 581–586.

REES, C. W., AND REARDON, L. V., 1945. Comparative yields of *Endamoeba histolytica*-organism *t* from soluble and insoluble ingredients of egg white in freshly prepared and stored medium, *Am. J. Trop. Med.* **25**: 109–114.

REES, C. W., AND REARDON, L. V., 1947. Studies on the growth requirements of metacystic *Endamoeba histolytica*, *J. Parasitol.* **33**: Suppl., 21.

REES, C. W., REARDON, L. V., AND JACOBS, L., 1941. The cultivation of the parasitic protozoa without bacteria. *Am. J. Trop. Med.* **21**: 695–716.

REES, C. W., REARDON, L. V., JACOBS, L., AND JONES, F., 1941a. Problems encountered in the growth of *Endamoeba histolytica* in cultures developed by micro-isolation, *Am. J. Trop. Med.* **21**: 567–578.

REES, C. W., REARDON, L. V., JOHNSON, E. M., AND MAYFIELD, F., 1945a. The influence of whole egg inoculum on the growth of *Endamoeba histolytica*-organism *t* in enriched egg white medium and in whole-egg dialysate medium, *J. Parasitol.* **31**: Suppl., 7.

REHWALD, F., 1926. *Starch-making and the manufacture of Dextrin, Starch, Sugar, Syrup, and Sugar Coloring*, Scott, Greenwood & Son, London, 264 pp.

SHAFFER, J. G., AND FRYE, W. W., 1948. Studies on the growth requirements of *Endamoeba histolytica* without viable bacteria. I. Maintenance of a strain of *E. histolytica* through one hundred transplants in the absence of an actively multiplying bacterial flora, *Am. J. Hyg.* **47**: 214–221.

SHAFFER, J. G., AND SIENKIEWICZ, H. S., 1952. Propagation of a strain of *Endamoeba histolytica* in tissue-bearing culture, *Science* **116**: 306–308.

SNYDER, T. L., AND MELENEY, H. E., 1943. Anaerobiosis and cholesterol as growth requirements of *Endamoeba histolytica*, *J. Parasitol.* **29**: 278–284.

STONE, W. S., 1935. A method of producing encystment in cultures of *Endamoeba histolytica*, *Am. J. Trop. Med.* **15**: 681–684.

WERKMAN, C. H., 1946. Manual of methods for pure culture study of bacteria. *Soc. Am. Bacteriologists*, **10**; Leaflet VI.

HEMATOLOGIC DIFFERENTIATION OF THE INTRAMUSCULAR DEVELOPMENTAL FORMS OF *TRYPANOSOMA CRUZI* CHAGAS[1]

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INTRODUCTION

Since observers have not used hematological criteria for differentiating the morphology of *Trypanosoma cruzi* Chagas, the writer has investigated the structure of this parasite from the viewpoint of a hematologist. Because the supportive film must be thin for proper cellular differentiation, blood smears and tissue contact preparations of intramuscular forms were used from white mice experimentally infected with NIH Brazilian trypanosomes. The results support the hypothesis of Mayer and da Rocha-Lima (1912, 1914) and Elkeles (1944, 1945, 1951) of a regressive development from trypanosomes to leishmaniform parasites and progressive differentiation of leishmaniform *Trypanosoma cruzi* into trypanosomes, indicating an intramuscular developmental cycle.

MATERIALS AND METHODS

Thirty-four male white mice, *Mus musculus*, were used for study of blood and tissue parasites. All mice were strain CC 1 from the California Caviary, Los Angeles. All inoculations were intramuscular into left and right gastrocnemius muscles for experiments 156 to 189. The mouse was placed in a hardware cloth cylinder, the hind limb pulled backward, hair clipped and the hypodermic needle introduced at a 45° angle to or crosswise of the long axis of the muscle. Some inoculations were made parallel to the two rami of the long axis of the gastrocnemius from the Achilles tendon area. This latter method reduced leakage of inoculum through the skin from tissue pressure.

The trypanosomes used in this investigation were the NIH Brazilian strain supplied by the Laboratory of Tropical Diseases, Microbiological Institute, National Institutes of Health, through the courtesy of Dr. Theodore von Brand and Eleanor J. Tobie. *Triatoma protracta* nymphs and adults were fed upon white rats infected with this strain of *Trypanosoma cruzi*. These bugs were fed on laboratory animals when needed to retrieve clear fecal matter containing metacyclic trypanosomes. Each sample was scanned under the microscope before inoculation to check the concentration of trypanosomes, although no counts were made. The inoculum of experiments 156 to 158, 166 to 168, 173 to 180, 181, 184 and 185 consisted of 0.05 ml. suspensions in sodium citrate solution of one to many fecal samples of adults or nymphs of experimentally infected *Triatoma protracta*. Experiments 159 to 165, 169 to 172, 182, 183, and 186 to 189 were inoculated with 0.05 ml. suspensions in sodium citrate solution of 2 drops of

[1] This research was supported by a grant from the United States Public Health Service.

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tail blood from mice with heavy blood parasitemias. Animals were sacrificed at different time intervals to check developing tissue forms.

Air-dried and heat-fixed tissue-contact preparations were made from blood and gastrocnemius muscles of experimentally infected white mice. All slides were stained with Jenner-Giemsa. The writer has used the term tissue impression previously for the technique used here (Wood, 1951a). Since the term connotes pressure to some investigators, the term tissue contact is a more accurate descriptive phase for the "touch system" used for trapping cells in the accumulated tissue surface film, avoiding as completely as possible any contact pressure between slide and tissue. This technique was also used by Mayer and da Rocha-Lima (1914).

Experimental intramuscular infection of white mice with NIH *Trypanosoma cruzi* revealed an 8-day incubation period as judged by initiation of blood parasitemia. Therefore, it was assumed that the parasite completed its regressive and progressive developmental cycle in that time. Experimental infections failed to produce sufficient numbers of parasites in gastrocnemius muscles during the first 8 days of the infection to permit following the complete cycle during that time. However, it was soon realized that the intense parasitemias which developed signified the reinvasion of the gastrocnemius as well as other muscles, especially heart, where the cyclical development was repeated. Tissue contact preparations revealed no parasites on the 2nd day (160, 161) and 4th day (166), leishmaniform stages on the 4th day (163) and 5th day (168, 174, 176) with regressive and progressive stages on the 6th day (170, 171), progressive leishmaniform on the 7th day (175), and mostly progressive leishmaniform, transition and trypanoform parasites on the 8th (152) and the 9th (185) days. Regressive and progressive leishmaniform parasites were found on the 8th day for experiment 186. In experiments 164 and 169, the mice were sacrificed on the 19th and 28th days after inoculation when the parasitemia indicated heavy intramuscular development and all stages of regressive and progressive parasites can be found in these preparations.

Counts of live trypanosomes were made under an 18 mm. circular coverglass with the high-dry objective as the parasitemia increased or decreased. After reaching 500 parasites per coverslip, counts were discontinued and the extent of the area surveyed was noted. With the high-dry objective used, 362 fields were counted in one coverage of an 18 mm. circular coverslip including complete half-fields at the edges. Relative concentration of trypanosomes was estimated from number of trypanosomes and number of microscopic fields counted to indicate the number of parasites per field.

The hematological nomenclature of Pappenheim and Hirschfeld (1920) is employed for describing cellular changes.

The criteria selected for cytological differentiation of developmental stages were: the degree of basophilia; the amount of volutinization and vacuolization of the cytoplasm; the shape, position and chromatic structure of the nucleus and kinetoplast; the structure of the flagellum; the presence or absence of an undulating membrane; and the shape of the parasite.

Regressive changes from trypanoform to leishmaniform parasites are indicated by decreased acidophilia and increased basophilia with apparent "thickening" of the cytoplasm; increased volutinization and vacuolization showing distinct alveolar structure; shifting of the nucleus from oblong to spherical form with increase in volume; lengthening of the kinetoplast from bluntly oval to triangular and rod form with increase in volume; reduction in length of the flagellum; disappearance of the undulating membrane; and change from elongate, wavy, ribbon body type to crescent shape with ends curving together or broadening and shortening to produce a spherical body form.

Dameshek (1947) points out that when blood cells stain blue it is because the cytoplasm has affinity for basic dyes and is itself acid and that these cellular changes may now be differentiated by chemical means. Jones (Dameshek's Morphologic Hematology, 1947) reports that the basophilic cytoplasm of blood cells is produced by variable concentrations of ribonucleoproteins. Eakin (1949) reports Brachet as observing that a rise followed by a decline in cytoplasmic basophilia represents synthesis and release or breakdown of ribonucleic acids so that cytoplasmic basophilia is indicative of the presence of ribonucleic acid. Apparently, in *Trypanosoma cruzi*, cytoplasmic basophilia increases as the parasite undergoes regressive changes from trypanoform to leishmaniform stages and decreases as it develops progressively to the mature trypanosome.

Progressive differentiation from leishmaniform to trypanoform parasites is indicated by decreasing basophilia and increasing acidophilia with apparent "thinning" of the cytoplasm; decreased volutinization and tendency for accumulation of the few remaining volutin granules between nucleus and free flagellum; disappearance of vacuolization; change from spherical nucleus to laterally compressed, elongate, bacilliform type with decrease in volume; change from rod-shaped kinetoplast to triangular form and eventually compressed oval shape with decrease in volume; lengthening of the flagellum; development of the undulating membrane over cytosome between flagellum and cell membrane; and change of spherical body form to flattened teardrop shape with cytoplasmic splitting and untwisting to the trypanoform parasite or simple elongation of the spherical body form with subsequent extension into the elongate, wavy, ribbon body form.

Evidences of degeneration, as contrasted with regressive changes, were: decreasing basophilia of the cytoplasm unaccompanied by acidophilia; disappearance of volutin granules; variable sized vacuolization of the cytoplasm; decreased size of the nucleus with more irregular shape; pycnosis of basi-chromatin and disappearance of oxychromatin and heterochromatin; and elongate, flattened, crescentic body form with disappearance of the flagellar apparatus.

Blood and tissue counts involved the following categories: C shaped and S shaped forms of regressive and progressive trypanosomes; regressive and progressive transition parasites; dividing, regressive and progressive leishmaniform parasites; slender C or S trypanoform parasites with or without extended protoplasmic caps; degenerating C and S forms and unclassified parasites.

OBSERVATIONS

The parasite introduced into the gastrocnemius muscle was the metacyclic form of *Trypanosoma cruzi* Chagas from the insect vector, illustrated in Plate 1, Figure 17. These slender parasites undergo regressive changes resulting in the formation of leishmaniform organisms which by binary fission eventually fill the areas occupied at their developmental sites.

Thus, the first stage of the regressive development, Plate 1, Figure 1, is characterized by the following cell changes: increase in volume of the cytoplasm resulting in thickening of the cytosome; shift of the nucleus from a slightly anterior to a central position, accompanied by a change from an elongated oval to an oval form; increased cytoplasmic basophilia; vacuolization of the cytoplasm; appearance of many volutin granules; and a compacting of the triangular-shaped kinetoplast which stains very densely, more black than purple. This intense staining of the kinetoplast may be an important cytochemical clue since it may be indicative of chemical changes in that organelle as basophilia is an indication of such changes in the cytoplasm.

The regressive trypanosome, Plate 1, Figures 1 and 2, continues to broaden posteriorly until the free flagellar end approximates the posterior protoplasmic cap enclosing the kinetoplast. At the same time the nucleus becomes more rounded, exhibiting finer clumping of the basi-chromatin with an increase in the parachromatin spaces. The spongioplasm of weakly basophilic or lightly acidophilic nature becomes restricted by the extensive, deeply basophilic paraplasm which spreads throughout the rounding cytosome. The organism appears to change from a laterally flattened, ribbon-like body pattern to an elongate half-moon shape and eventually a rounded form. The nucleus increases in volume and the flagellar apparatus loses its undulations. Broad regressive trypanosomes are illustrated for Brazilian parasites in Figures 6 to 22 and the lower parasite of Figure 27 of Plate 10 by Chagas (1909), the lower parasite of Figure 2, Plate 8, by Mayer and da Rocha-Lima (1914), and Figures 1, 6, 10 and 11 of Plate 1 by Nägler (1913); for Argentine parasites in Figures 1 to 8 and 16 of Plate 3 by Niño (1928); and for California parasites in Figures 8, 9, 10, 11, 15, 16 and 17 of Plate 2 by F. D. Wood (1934). Rey Matiz (1941) noted that Colombian trypanosomes always showed vacuolated cytoplasm, indicating broad regressive types.

As the cytosome becomes more rounded, developing a shallow semicircular U or V-shaped indentation between the two extremities which are now merging, the regressive transition form, Plate 1, Figures 3 and 4, can be recognized. This parasite shows deep basophilia, vacuolization, and volutinization of the cytosome. The nucleus is rounded, with finer, more dispersed chromatin masses and the kinetoplast is small, like that of the regressive trypanosome with a similar intense denseness manifested by its deep purplish-black color. The constant appearance of vacuolated areas near the kinetoplast suggests a possible role in volume regulation associated with assumption of the flattened circular form. Some parasites show much thinner flagellar axonemes suggesting resorption of that organelle until the typical leishmaniform organism is formed. The only illus-

tration of this stage previously described is that of Niño (1928) in Figure 31 of Plate 3 for Argentine *Trypanosoma cruzi*.

Figure 4 of Plate 1 is characterized by large volume, deep basophilia, conspicuous volutinization and vacuolization and a rounded nucleus. However, the most distinctive feature is the deep purplish-black color of the kinetoplast which is found in all the cells of the regressive series including Figure 5.

The regressive leishmaniform stage of Figure 5 is separated from other parasites of the regressive series by the compact, dense, purplish-black kinetoplast, vacuolated basophilic cytoplasm and volutin granules scattered through the cytoplasm. Most of these organisms show lighter basophilia than other stages of the regressive series.

The dividing leishmaniform parasites of Figures 6 and 7 show an abrupt change in structure of the kinetoplast from the preceding members of the regressive series. The large, voluminous, bean-shaped kinetoplast which is preparing to divide has a deep black-purplish color which is characteristic of it in the progressive developmental series, Figures 8 through 13, 15 and 16. The cytoplasm is less basophilic with few or no vacuoles. Volutin granules are fewer and are found most frequently anterior to the nucleus. The nucleus shows a finer meshlike network of chromatin. After division, the leishmaniform flagellum begins to grow with the undulating membrane appearing very early as in Figure 8.

The progressive leishmaniform parasite of Figure 8 arises from the large dividing leishmaniform stages. They have lightly basophilic cytoplasm with no vacuoles and few volutin granules. The kinetoplast is large and bean-shaped, staining blackish purple with Jenner-Giemsa. The flagellum is short or long, attached only at its base near point of origin at the kinetoplast with or without a short undulating membrane. Such progressive leishmaniform parasites have been illustrated for Brazilian *Trypanosoma cruzi* by Vianna (1911) in Figure 2 of Plate 9 and Delanoë (1912) in Figures 1 to 7.

The progressive transition parasite of Figure 9 is characterized by the elongating flagellum with developing undulating membrane attached to the curving cell membrane of the circular cytosome. The cytoplasm is less basophilic with few or no volutin granules. Some slides failed to demonstrate the undulating membrane clearly but by prolonging the Giemsa staining 20 to 25 minutes, the membrane becomes distinctly pink. There is some variation in length of the free flagellum since cells are found with long or short axonemes attached to the cytosome by short undulating membranes, indicating that the axoneme may extend itself more rapidly than the undulating membrane. However, a continuous series of stages from short to long flagellum with attachment to the cytosome by progressively longer undulating membranes can be found in progressive development. Trypanosome formation from this progressive transition parasite may occur either directly, by elongation to the trypanosome form (Figures 9, 15, 16, and 12) or indirectly, by transition formation and untwisting to the trypanosome form (Figures 9, 10, 11 and 12) as previously described for Arizona *Trypanosoma cruzi* (Wood, 1951a). The indirect development is more commonly seen in the Brazilian material.

As the flagellar encirclement of the progressive transition parasite continues, the body flattens so that the flagellum is oriented along the greatest circumference of the cytosome. Thus, as the arc of growth is completed, the free flagellum approximates the kinetoplast. The cytoplasm becomes acidophilic in the area between kinetoplast and base of the free flagellum, developing a U or V-shaped indentation thus freeing the future anterior end of the parasite. When the free flagellar end untwists in either direction away from its original plane of cytosomal encirclement, the progressive trypanosome is formed. Progressive transition Brazilian parasites have been shown by Chagas (1909) in Figure 30 of Plate 10, Mayer and da Rocha-Lima (1914) in Figures 20, 27 and 28 of Plate 8 and Delanoë (1912) in Figures 9, 10, 11 and 18. Reina Guerra (1939) illustrated a progressive transition form as the 6th organism from the right in Figure 14 from El Salvador infections in white rats.

As the intermediate-sized progressive trypanosome (Figures 12 and 13) becomes more slender, the nucleus remains slightly anterior, changing from a round to an oval shape and finally an elongate, laterally compressed oval form. At the same time the cytosome undergoes reduction in volume and basophilia with development of spongioplasm at the expense of paraplasm. In most parasites, a few volutin granules persist, aligning themselves characteristically between the nucleus and the future anterior end of the trypanosome. Soon after untwisting, the developing acidophilia characteristic of the mature progressive trypanosome appears. It begins in the central areas of the cytosome anterior to the kinetoplast and spreads radially towards the periphery of the cytoplasm. Early progressive Brazilian trypanosomes are illustrated by Chagas (1909) in Figure 29 of Plate 10 and by Delanoë (1912) in Figures 12, 13, and 19 to 21.

The progressive trypanosomes (Figures 12 and 13) arising by direct or indirect development outnumber regressive parasites (Figure 14) in tissue slides. They are variable in form at first as they change from broad, slowly motile forms to narrow, actively motile trypanosomes. They originate mostly from medium-sized progressive transition parasites by untwisting (Figures 9, 10, 11, 12) although some arise by elongation (Figures 9, 15, 16, 12). With reduction of the cytoplasm and compacting of the nucleus and kinetoplast, the narrow, ribbonlike body of the mature parasite is formed. The smaller number of progressive trypanosomes in the peripheral blood may be an expression of the influences of the chemical environment causing rapid cytoplasmic change from acidophilia to basophilia as the parasites progress from the internal to the peripheral circulation.

Some tissue trypanosomes have very large, rounded, triangular or heart-shaped kinetoplasts similar to those of leishmaniform stages. These are found in groups near large leishmaniform parasites from which they originate by direct development. The forward growth of the flagellar apparatus pulls the cytoplasm with it, including the nucleus of the developing parasite so that, as it stretches out, the kinetoplast remains at one end of the cytosome and the free flagellum at the other with the nucleus near the center.

Correlated with these tissue developmental stages are the blood forms (Plate

1, Figures 14, 18 and 19) which are also found in the tissues. The long, slender, progressive trypanosome (Figure 19) showing an anteriorly displaced nucleus has two types, with or without extended posterior protoplasmic caps. Parasites similar to Figure 16 of Plate 1 of Wood (1951a), or having a teardrop form, give rise to the elongate slender trypanosome. They are produced from large leishmaniform parasites as a result of tissue pressure at the developmental site or chemical suppression of development or both, delaying the separation between future anterior and posterior extremities of the parasite. Some forms with irregular protoplasmic extensions beyond the kinetoplast, associated with both the unseparated and slender forms as Figure 19 here, suggest such a formation. These parasites by the same processes as described above under regression in the trypanosome form, may develop into regressive trypanosomes. Long, slender, progressive Brazilian trypanosomes have been illustrated by Chagas (1909) in Figures 23 to 26, and 28 and the upper parasite of Figure 27 of Plate 10, by Mayer and da Rocha-Lima (1914) in the upper parasite of Figure 2, Plate 8, by Delanoë (1912) in Figure 14 and by Nägler (1913) in Figures 2, 3, 8, 9, and 12 of Plate 1. F. D. Wood (1934) illustrated photomicrographically slender California trypanosomes in Figures 12 and 13 of Plate 2.

The retention of the pear-shaped body form by developing long slender progressive parasites eventually leaves a broad mass of protoplasm forward and lateral to the kinetoplast and anterior to the first curve of the flagellum. This cytoplasmic mass in some specimens appears to slip beyond the kinetoplast while the parasite extends itself, thus producing at first an irregular cytoplasmic cap which by subsequent motion of the parasite becomes the conspicuously long, narrow, cone-shaped mass. This cytoplasmic cap is characteristic of the elongate slender trypanosome which, as a result of this extended form and rhythmic continuous wriggling, is able to progress through the blood with rapid rhythmic twisting of flagellum, undulating membrane and cytosome. If this protoplasmic mass remains anterior to the kinetoplast, the nonextended slender form is produced without conspicuous cytoplasmic cap.

The short, slender progressive trypanosome, Figure 18, originates from small leishmaniform parasites by direct development as shown by the presence of intermediate stages between these two parasites. The distinctly acidophilic or lightly basophilic appearance of the cytoplasm accentuates the elongate, oval, laterally flattened nucleus. F. D. Wood (1934) illustrates photomicrographically a short, slender, progressive, California trypanosome in Figure 14 of Plate 2.

The most common trypanosome of the blood in these experimental infections was the large, broad regressive trypanosome with slightly anterior oval nucleus shown in Figure 14. It is easily distinguished from all other blood forms by its laterally flattened oval nucleus and deeply basophilic cytoplasm which at first shows spongioplastic acidophilic or light basophilic areas which are replaced by a deeply basophilic spongioplasm with vacuoles and volutin granules thus transforming it to the regressive trypanosome described above (Figure 1). The oval nucleus exhibits scattered clumps of basichromatin surrounded by narrow zones of oxychromatin with scattered parachromatin spaces. The shift in the

nucleus from a central to a slightly anterior position in *Trypanosoma cruzi* is evident during aging of regressive trypanosomes as well as development of progressive trypanosomes. The early regressive forms tend to show slight anterior displacement (Figure 1) but the older regressive forms show marked anterior displacement (Figure 2). This anterior nuclear displacement is never as great in *Trypanosoma cruzi* as it is in *Trypanosoma vespertilionis*. Examples of broad regressive *Trypanosoma cruzi* with anteriorly displaced nuclei are shown for Argentina parasites by Niño (1927) in the cat, Mazza and Romaña (1934) in man, Mazza and Driollet (1935) in the armadillo, Mazza, Miyara and Sanjurjo (1936) in the fox and ferret (*Grisonella*), Mazza and Benitez (1937) in man, Mazza and Jörg (1939) in man, Mazza (1940) in the squirrel and ferret (*Tayra*) and Manso Soto (1948) in man; for Brazilian parasites by Dias (1936) in man, armadillo and guinea pig, Pessôa, Coutinho and Mareira (1941), Dias (1942) and Dias, Laranja and Nobrega (1945) in man; for Chilean parasites by Bertin Soto (1940) in man; for Colombian parasites by Rey Matiz (1941) in white mice; for parasites from El Salvador by Reina Guerra (1939) in white rats; for parasites from French Guiana by Floch and de Lajudie (1941) in the armadillo; for Panamanian parasites by Robertson (1929) in the opossum, and for Texan parasites by Packchanian (1939) in white mice.

All these blood trypanosomes occur in a C or S form in the blood or tissue preparations as noted by Chagas (1909). Regressive trypanosomes are most fragile as noted below. Niño differentiated O, U, S, hook (=C) or question mark forms of Argentine blood flagellates. F. D. Wood (1934) noted U and S-shaped California trypanosomes while Rey Matiz (1941) noted U and C-shaped Colombian parasites.

Transition trypanosomes between all blood forms suggest a developmental pattern of all types to broad regressive trypanosomes since the short and long slender forms develop broader, basophilic cytosomes to become regressive forms. As the parasitemia increases, some of these regressive forms degenerate as evidenced by development of a pale blue cytoplasm without vacuoles or volutin granules, a constricted oval kinetoplast, and a smaller rounded nucleus with broad, amorphous chromatin clumps separated by acidophilic nucleoplasm without parachromatin spaces.

The first sign of development to regressive trypanosomes from short, slender or broad blood trypanosomes is a bulging of the cytosome anterior to the kinetoplast and at the point of contact with the undulating membrane. This is followed by increasing basophilia, increased vacuolization and volutinization, rounding of the nucleus and assumption of a crescentic body form.

As the parasitemia appears in mice, the long, slender progressive trypanosomes are most noticeable especially in fresh blood counts. These trypanosomes swim rapidly through the microscopic field, progressing from one area to another, in sharp contrast to the more stationary tendencies of other blood trypanosomes. However, since these parasites were noted adhering to the red corpuscles, they are probably positively charged as noted by Fairbairn and Culwick (1946) for slender forms of African trypanosomes. As the short, slender progressive and

broader regressive trypanosomes increase in numbers only a few long, slender progressive forms persist. Counts of fresh blood revealed the following percentages of long, slender progressive trypanosomes: 8th day 33.3 (189); 10th day 11.7 (164); 11th day 14.2 (162), 2.7 (165); 12th day 2.0 (164), 7.1 (187), 30.3 (188), 27.3 (189); 13th day 10.6 (182), 0.0 (183); 14th day 33.3 (181), 9.0 (184), 26.3 (187), 41.4 (188); 15th day 6.7 (162), 3.5 (165), 2.2 (183); 16th day 30.9 (181), 1.2 (183), 12.1 (188); 17th day 1.2 (162 and 165); 20th day 8.9 (178); 22nd day 0.4 (178); and 24th day 1.5 (178). The numbers in parentheses indicate the experimental animal. These counts are made rapidly to avoid counting any one trypanosome twice. If the preparations stand a few minutes, the positively-charged, slender forms adhere to the negatively-charged red corpuscles in sodium citrate solution thus checking their progressive movements.

As the infection reaches its peak in the blood, the broader regressive trypanosomes predominate. If the animal dies, the regressive trypanosomes are most numerous with small numbers of progressive short and long, slender trypanosomes continuing to appear until death as noted in experiment 181. During later stages of heavy parasitemias, regressive trypanosomes predominate. As host resistance increases, as in experiments 162 and 165, and the parasitemia decreases, long, slender progressive parasites become more noticeable. In experiment 162 on the 37th day, two of 13 trypanosomes counted under an 18 mm. circular coverglass were long, slender progressive parasites. In experiment 165, four of 59 parasites on the 33rd day and three of 18 on the 35th day were long, slender progressive forms.

Counts of 1000 parasites on each of 18 air-dried (C) or heat-fixed (H) smears from 3 different animals revealed 6 with fragility factors (See Wood, 1951b) of 0.4% to 9.9%, averaging 2.9%. One count was on the 21st day (162C), 2 counts on the 29th day (162C and 165C), one on the 35th day (162C) and two on the 38th day (181C and 181H). The nearest average percentages for the 6000 parasites counted were: regressive trypanosomes 92.8, C forms 79, S forms 13.8; progressive short slender trypanosomes 1.7, C forms 1.4 and S forms 0.3; progressive long slender trypanosomes 0.4; degenerating parasites 1.6; and transition parasites 0.2. With fragility factors from 11.2% to 90.4%, the percentages for degenerating, transition, long slender and short slender parasites are similar to those noted above, the reduction in numbers affecting chiefly the regressive C and S form trypanosomes. In the 12 counts tabulated with high fragility factors, the counts of regressive trypanosomes varied from 8.2% to 85% with the C forms averaging 3 times as many as the S forms. The counts reveal that the C forms are more fragile than the S forms.

Two counts of 1000 parasites each from the first tissue-contact slides of left and right gastrocnemius from experiment 164 sacrificed on the 19th day revealed the following parasites: dividing leishmaniform 10.4%; regressive leishmaniform 2.0%; progressive leishmaniform 23.3%; regressive trypanosomes, C and S forms, 0.1% each; and progressive trypanosomes, C forms 13.7% and S forms 7.4%. The fragility factor averaged 13.3% on these two slides in the area surveyed.

DISCUSSION

The chief advantage of the tissue-contact method of slide preparation is that all types of parasites in the tissue surface at time of contact will be represented. If there are many parasite remnants, i.e., a high fragility factor (Wood, 1951b), interpretations are limited to those forms preserved and this must be related to interpretations of cyclical development. Therefore, one can determine immediately with dry fixed material whether the preparation is suitable for studying cyclical development. With wet fixed smears, it is not possible to know if all stages are represented in the liquid film after fixation since organisms may rupture and disappear without the preparator being aware of it.

In comparing results of other workers with white mice, one important variable is the inoculative method. As indicated previously (Wood, 1949a), tissue inoculative methods involve a pressure factor which may destroy parasites before they can invade tissues. This can be avoided by use of eye, mouth, nose or peritoneal cavity as the point of contact for living trypanosomes. However, since these experiments were primarily to study muscle dwelling forms, direct inoculations into the gastrocnemius were made. The few developing parasites found in experiments 163(1), 167(183), 168(2), 174(1), 175(3), 176(1), and 185(9), as well as none in 166, 170 and 171, may represent high host resistance, a pressure factor, lack of sufficient time for development, escape into other muscles or a combination of these factors.

Collier, Fulton and Innes (1942), studying 250 white mice infected with a Brazilian strain, noted trypanosomes in the blood 2 to 12 days after intraperitoneal, subcutaneous or retro-orbital inoculation. They recognized a negative blood phase, except by intravenous inoculation, a period of slowly increasing parasitemia, a phase of acute pathological changes, and a chronic state when the patent blood infection fades. They noted that mice may die at any stage from trypanosomal septicemia or concomitant intercurrent disease, while some were not apparently ill. A marked edema lasting one week and occurring 2 or 3 weeks after inoculation was the most conspicuous sign of infection in most of their mice but did not extend into the lower leg muscles. No gelatinous infiltrations were seen in any of the gastrocnemius muscles here studied. Edema of other areas of the body was observed in experiments 159(26th day), 164(19th day), and 172(15th day).

Since Hauschka (1947) has shown that male mice are more susceptible to the "B" strain used here, only males were infected. Strain characteristics have been studied for this Brazilian source of *Trypanosoma cruzi* by Hauschka (1949) and Goble (1951). Hauschka (1949) reported a constant virulence, definite affinity for heart muscle and no seasonal rhythm when using inocula of 500,000 blood trypanosomes per mouse. Goble (1951) reported variation in virulence with smaller inocula (15 million culture forms per mouse) but more constant virulence with higher inocula (30 million culture forms). This difference in virulence may reflect fewer surviving metacyclic trypanosomes in the inoculum from cultures.

In the writer's experimental animals, developmental forms were found in heart muscle on the 19th day (164, 173) and 26th day (159).

Although continuous counts were not made by the writer, each infection which was allowed to develop a heavy parasitemia produced 4 to 5 parasites per microscopic field. This concentration of trypanosomes was reached in these experiments on the following days: 14th(164), 16th(183), 17th(162, 165), 20th(179, 181, 188) and 28th(169).

The conclusions reached here are based upon survey of at least 41,222 stained parasites of *Trypanosoma cruzi* Chagas. Most of these specimens were from blood smears and muscle tissue-contact preparations but a few were from heart and liver contact slides. The same progressive developmental cycle seen here in striated muscle was found also in heart muscle.

After tissue-contact preparations were made with left and right gastrocnemius muscles, the tissue was teased in a large drop of sodium citrate solution and the parasites counted under an 18 mm. circular coverglass. In one such preparation from experiment 181 after death of the animal on the 38th day, a double parasite was seen swimming among agglomerated masses of trypanosomes. The two trypanosomes were joined by their posterior extremity with a single kinetoplast similar to Figure 3 of Wood (1949b) from *Triatoma* feces. The bodies of the two trypanosomes formed an angle of about 45°, with a single protoplasmic cap enveloping a very large kinetoplast.

As noted above, the writer distinguishes three blood trypanosomes, short, slender progressive; long, slender progressive with or without extended posterior protoplasmic caps; and broad regressive parasites. Chagas (1909, 1911) differentiated slender and broad trypanosomes, believing they represented males and females respectively. These are trypanosomes of different sizes which originate from variably sized, leishmaniform and transition parasites as indicated above. No evidence that these represent sexual forms could be found by the writer. Chagas also discussed intracellular forms in red blood corpuscles which could not be found here. Chagas considered the long, slender progressive trypanosomes as "young" forms, which they are in the sense that they have recently entered the blood from the tissues. They develop into regressive trypanosomes in the blood. Chagas described the regressive process (retrogression of Elkeles, 1944) perfectly in discussing schizogony but nothing found here would support his concept of an intracellular schizogony. Forms found here similar to Figure 3 of Plate 1 (Wood, 1951a) are interpreted as representing the multimastigont condition resulting from delayed cytosomal division as indicated for other flagellates by Kirby (1944).

The many examples of trypanosomes superimposed on red corpuscles observed by Chagas (1909) were not seen with the NIH Brazilian strain. Broom, Brown and Hoare (1936) showed that blood forms of one strain of *Trypanosoma cruzi* were negatively charged and pointed out that such forms remain free of negatively-charged red corpuscles. However, positively-charged *Trypanosoma brucei* showed parasites adherent to the red corpuscles. Fairbairn and Culwick (1946)

pointed out that in *Trypanosoma rhodesiense* there were positively and negatively-charged variants and red corpuscle contacts were characteristic of the positively-charged, longer trypanosomes of any type. Probably Chagas (1909) was working with a strain of positively-charged *Trypanosoma cruzi* during some phase of its cycle.

Chagas (1911) recognized the possible incompatibility of the blood as a medium for developing regressive trypanosomes and postulated a trypanolytic substance which determined the number of blood trypanosomes. Mazzotti (1940) also believes the decrease in blood trypanosomes is due to a trypanocidal agent. There is certainly some suppressive agent or physiological process which causes the trypanosomes to disappear from the blood of most animals in nonlethal infections.

Vianna's (1911) Figure 4 of Plate 14 showing progressive developing parasites in guinea pig muscle suggests direct development by elongation under pressure of the muscle tissue. Mayer and da Rocha-Lima (1914) suggest that the strong edema in skeletal muscle of mice may be a factor in the appearance of spindle-shaped leishmaniform parasites. Their Figure 2 of Plate 9 shows the elongated leishmaniform parasite in smooth muscle, suggesting direct development by elongation as illustrated in Figures 8 to 15 of Plate 8. This could also determine the teardrop transition forms from which elongated slender trypanosomes develop as noted here. The slender forms appearing first in the circulation may indicate a greater pressure factor during early muscular development. If these areas are rounded, the parasite retains a rounded form, developing indirectly as previously described. However, if pressure factors result in the parasite adapting itself to oval or elongated areas within the muscle, the organism elongates, developing into trypanosomes by the direct method.

Mayer and da Rocha-Lima (1912, 1914), using a human Brazilian strain obtained in a guinea pig, studied the developmental pattern in mice. They noted the broad and slender parasites in the blood and that the slender forms appeared earlier than the broad forms which predominate. They believed the slender forms originated from recently arrived trypanosomes undergoing schizogony in the blood. They rarely reported leishmaniform parasites in the peripheral blood in heavy infections of monkeys, guinea pigs and mice. Their concept of the muscular development was penetration of the tissue by the trypanosome, rounding of the body with reduction of flagellar apparatus, appearance of large leishmaniform cells with or without flagella either intercellularly or intracellularly, binary fission of leishmaniform stages, flagellar growth over rounded parasites and unrolling (*aufrollung*) to form trypanosomes. They recognized leishmaniform parasites of small and large size and spindle forms, the latter being rare. They believed the spindle forms were induced by muscular pressure and developed into crithidias, then trypanosomes, by migration of the kinetoplast, in contrast to the writer's belief that this is direct development by movement of cytoplasm and nucleus (Wood, 1951a). They believed the small leishmania was a transition stage between large leishmanias and trypanosomes in contrast to the writer's concept of the two sizes producing two types of blood trypanosomes. They noted

the similarity in size and structure of individuals from any one group of developing parasites and the greater abundance of large leishmanias in their preparations.

Study of the color plates of Mayer and da Rocha-Lima (1914) reveals forms illustrating direct development by elongation in Figures 8 to 15 and indirect development in Figures 24 to 32 of Plate 8. Figure 2 of Plate 9 suggests direct development in smooth muscle while Figure 3 suggests indirect development in heart muscle.

M. and P. Delanoë (1912) studied Giemsa-stained parasites from mouse muscle and concluded that the pyriform-shaped (fusiform) or elongated leishmaniform cells were transition stages between rounded leishmaniform and trypanoform parasites and that there was no dimorphism in development as noted by Mayer and da Rocha-Lima (1912). They emphasized the dimorphism of blood trypanosomes and were the first to express the belief that these were related by intermediate forms. They believed the parasites developed through leishmania, leptomonas, crithidia and trypanosome stages in muscle tissue as they do in the insect vector. The leptomonad forms, Figures 8 and 9, and crithidias, Figures 10 and 11, are classified here as elongated, fusiform, leishmaniform parasite (8) of direct development and progressive, transition parasites (9, 10, 11) of indirect development. Figures 12 and 13 are progressive trypanoform parasites of direct development. Figures 15 to 21 illustrate some very interesting parasites from blood of heavily infected mice. Figure 15 is a nonflagellated leishmaniform parasite while Figures 16 and 17 are dividing leishmaniform stages. Figure 18 is a progressive transition form while Figures 19 to 21 are early progressive trypanoform parasites which have recently been formed by direct development. They observed developmental forms in red corpuscles (no figures), mononuclears, macrophages (Figures 26 and 27) and leucocytes as judged by the perfect preservation of intracellular leishmaniform stages. They did not find dividing trypanosomes in the blood although they remarked about the promitotic nucleus in Figure 1 of Plate 13 of Chagas' (1909) paper.

Nägler (1913) studied infections with a Brazilian strain in mice, rats and guinea pigs. He interpreted Chagas' sexual forms as variations of one type and reported finding transition forms between narrow and broad trypanosomes. He also found asexual division stages in the blood and peritoneal fluid of mice, indicating binary fission. No division forms of this type were seen by the writer on the stained slides studied, but one fresh preparation revealed a trypanosome in binary fission as noted above.

Hartmann (1910, 1917), studying Giemsa preparations made by Carlos Chagas, claims to have seen a true schizogony in the guinea pig lung. He claims origin of trypanosomes in an aflagellate condition within endothelial cells or intercellularly with large numbers of leishmania parasites developing from them. The writer believes that the text figure (1910) shows a macrophage with ingested leishmaniform parasites, a few of which are being destroyed. The figures for the 1917 note show portions of macrophage cytoplasm (1 and 2) or entire cells (3, 4 and 5) with leishmaniform parasites in various stages of disintegration. The

writer has seen bits of cytoplasm containing leishmaniform parasites in muscle contact preparations very similar to Hartmann's figures. Elkeles (1944) supports this concept of schizogony, citing his culture experiments to support this. The writer's observations agree with the interpretations of Wenyon (1926) concerning Hartmann's conclusions.

Wenyon (1926) figures very clearly both direct development in numbers 10 through 15 and indirect development in numbers 1 through 9 in Figure 207 from an original preparation made by Dr. E. Tejera.

Niño (1928) made a very extensive study of Argentine *Trypanosoma cruzi* in the blood and tissues of mice. Although he called Figures 1 to 3 of Plate 3 "trypanosomas jóvenes, formas machos de Chagas", they are actually broad regressive trypanosomes as indicated by the cytoplasmic basophilia and oval-shaped nuclei neither of which are characteristic of progressive slender parasites. An exceptionally interesting parasite is Figure 31, Plate 3, which portrays a regressive transition form as evidenced by the loss of the flagellum, spherical nucleus and vacuolization and volutinization of the cytoplasm.

Dias (1934), working with dogs, cats and guinea pigs, distinguished slender and broad forms, considering the latter adults of the slender trypanosomes, thus agreeing with the interpretations of Brumpt (1949). He found the slender forms rare in the initial blood phase but more numerous later. In kittens, after 24 hours, he found trypanosomes with volutin granules which he considered degenerating forms. As noted here, volutin granules increase in regressive parasites and decrease in progressive stages but may be present constantly. In infections of dogs, he found no initial blood phases after inoculation with *Triatoma* feces containing metacyclic trypanosomes. The writer's mice showed no trypanosomes in the blood until 8 days, agreeing with Dias' 8 to 11 day incubation period. In experimental infections with blood inoculum, Dias found an initial blood phase, noting also many slender forms appearing during the early phases of infection. Dias' concept of trypanogenesis was that trypanosomes enter the tissues, divide as leishmanias, pass through a short crithidial stage and become trypanosomes. He never noted leishmaniform parasites in the blood.

Elkeles (1944, 1945, 1951) has studied Argentine sources of *Trypanosoma cruzi* very thoroughly in mice. He describes a process of "retrogression" preceding multiplication, essentially as follows: decreased length and increased width and rigidity of the blood trypanosome; displacement of the kinetoplast from a peripheral to a more central position; swelling of cytosome with disappearance of flagellum and undulating membrane; appearance usually of a large vacuole near the kinetoplast; diminution of refractive power of parts of the parasite; and tendency of the parasites to collect in groups. As noted above, the writer prefers the term regression for this process which, although it may start in the blood, is completed in the tissue and was studied by the writer chiefly in the blood and striated muscle. Since Elkeles used wet fixation, his material is not strictly comparable to that of the writer. However, there are so many similarities between his results and the writer's that there is no doubt as to the com-

mon identity of the process. The air-dried or heat-fixed preparations of the author did not reveal a large vacuole but many small ones as characteristic of regressive stages, plus increased basophilia and volutinization, producing a less motile, more rounded parasite. The changes in the kinetoplast as described by Elkeles are identical to those observed in the writer's material.

Elkeles concludes that the leishmaniform parasite has two methods of change, "one toward the crithidia and the other toward the trypanosome." He indicates that the trypanosome develops in vertebrates from round forms without a crithidial stage. He uses the term unrolling for this process, following Mayer and da Rocha-Lima (1914), in contrast to an actual untwisting of the progressive transition parasite as shown by the writer for Arizona and Brazilian parasites. This process is identical to that described by Wood (1951a) in indirect development of Arizona trypanosomes and verified again here. Elkeles agrees with Mayer and da Rocha-Lima that the small leishmania or microleishmania is a transition form between large leishmanias and trypanosomes, in contrast to the writer's findings that the small leishmaniform parasite gives rise to the short slender trypanosomes of the blood.

Despite the statement of Elkeles at the beginning of the preceding paragraph, he considers the findings of Mayer and da Rocha-Lima (1914) illustrated in Plate 8, Figures 8 to 15 as "retrogression" even though they clearly agree with the writer's direct developmental cycle as illustrated with Arizona parasites (Wood, 1951a) as well as here with Brazilian material. Figures 8 to 15, Plate 8, of Mayer and da Rocha-Lima do not fit the writer's criteria for regressive stages but clearly represent progressive parasites transforming directly to trypanosomes. However, it is easy to see how crithidiform parasites could be interpreted from this material if we assume that the kinetoplast is stationary and the flagellar apparatus grows from it along a narrow wavy line over the cytosome. If soft clay is used to mold this developmental form, then dropped at various angles on the table as from a slice of tissue, one sees that, depending on contact position, sometimes it looks like a leptomonad with short flagellum or a crithidia if the undulating membrane has appeared. Since there is some tension created at the surface along the line of flagellar growth, not only the growth itself but also the movement of the flagellum and developing undulating membrane will tend to pull out or extend the cytosome and the nucleus will be pulled along with it. Thus, by a process of simple extension of the parasite's body, aided by edematous or muscular pressure or both, elongated fat or slender trypanosomes could result.

The vertebrate developmental cycle depicted by Brumpt (1949) in Figure 175 would be modified as follows: parasite number 5 would represent a transition form between 4 and 9, indicating direct development, while 7, 8, 6 and 9 would show maturing of blood forms changing from long slender progressive trypanosomes (7, 8) to broad regressive trypanosomes (6, 9). In a smear made by Dr. E. Téjera from cardiac muscle of a rat infected with Venezuelan parasites, one can identify easily all blood types as described here for a Brazilian strain (Brumpt,

1949, Figure 177). Progressive, slender trypanosomes, without extended protoplasmic caps, are shown as numbers 9, a progressive, slender trypanosome with extended protoplasmic cap is shown as number 8, a progressive, short, slender trypanosome is shown on the right middle edge of the illustration, while number 7 represents a regressive trypanosome.

SUMMARY

Metacyclic *Trypanosoma cruzi* transforms in muscle tissue to a regressive trypanosome which broadens and thickens while changing from a ribbonlike to a crescentic body form, developing many volutin granules and vacuoles. The elongated, oval, laterally compressed nucleus becomes oval and then round while the oval kinetoplast becomes triangular. Disappearance of the trypanosome shape results in a rounded, regressive, transition parasite which, by shortening or disappearance of the flagellum accompanied by development of the rod shaped kinetoplast, becomes the regressive leishmaniform parasite.

Large size of all organelles characterizes the dividing leishmaniform stage. The reduction of volutin, vacuoles and cell volume, with retention of rod-shaped kinetoplast and round nucleus characterizes the progressive leishmaniform parasite. The progressive transition shows an oval kinetoplast and enveloping flagellum with undulating membrane forming. If elongation occurs, the parasite, by flow of the cytoplasm and nucleus along the line of growth of undulating membrane and flagellum, transforms into the short, stout trypanoform organism. If flagellar growth continues over the circular, progressive, transition cytosome, a V-shaped indentation appears between the base of the free flagellum and kinetoplast area and the parasite untwists becoming a stout, progressive trypanoform parasite.

These progressive trypanoform parasites, by subsequent slimming of the body, elongation and compression of the nucleus, and condensation of the kinetoplast, become the progressive trypanosome which on arrival in the peripheral blood becomes the broad, deeply basophilic regressive trypanosome with anteriorly displaced nucleus. Since leishmaniform stages differ in size, small forms develop into small, progressive, transition and eventually short, slender, progressive trypanosomes. Large, leishmaniform parasites elongate under tissue pressure, resulting in long, slender, progressive trypanosomes. Small, intermediate and large trypanosomes, under the changing chemical environment of the hosts' blood, transform into regressive trypanosomes which may reinvade the tissues and repeat the cycle.

ACKNOWLEDGEMENTS

The writer wishes to express appreciation to Dr. Fae D. Wood for her constructive criticisms of this manuscript and her assistance in arranging and mounting the plate.

REFERENCES

1. Bertin Soto, V. 1940, Consideraciones sobre la epidemiología de la enfermedad de Chagas en Chile y su profilaxis, *Bol. Med. Social*, No. 77, 1–82.
2. Broom, J. C., Brown, H. C. and Hoare, C. A. 1936, Studies in microcataphoresis. II. The electric charge of haemoflagellates, *Trans. Roy. Soc. Trop. Med. & Hyg.* **30:** 87–100.
3. Brumpt, E. 1949, *Précis de Parasitologie*. In 2 volumes. 6th Ed., Paris, Masson et Cie, Éd., 1–2138.
4. Chagas, C. 1909, Nova tripanosomiaze humana. Estudos sôbre a morfologia e o ciclo evolutivo do *Schizotrypanum cruzi, n. gen., n. sp.*, agente etiológico de nova entidade morbida do homem, *Mem. Inst. Oswaldo Cruz* **1:** 158–218.
5. ——— 1911, Nova entidade morbida do homem. Resumo geral de estudos etiológicos e clínicos, *Mem. Inst. Oswaldo Cruz* **3:** 219–275.
6. Collier, H. O. J., Fulton, J. D. and Innes, J. R. M. 1942, The oedema of mice infected with *Trypanosoma cruzi*, and the accompanying pathological lesions, *Ann. Trop. Med. & Parasit.* **36:** 137–150.
7. Dameshek, W. 1947, *Morphologic Hematology*. New York, Grune & Stratton, 1–200.
8. Delanoë, M. and Delanoë, P. 1912, À propos du *"Schizotrypanum cruzi"*, *Bull. Soc. Path. Exot.* **5:** 599–602.
9. Dias, E. 1934, Estudos sôbre o *Schizotrypanum cruzi*, *Mem. Inst. Oswaldo Cruz* **28:** 1–110.
10. ——— 1936, Xenodiagnóstico e algumas verificações epidemiológicas na moléstia de Chagas, *9. Reunión Soc. Argent. Patol. Reg.* (Mendoza) **1:** 89–119.
11. ——— 1942, Apêlo aos clínicos do interior para a colaboração no estudo da doença de Chagas, *9. Hospital* **21:** 1–6 (Reprint).
12. ———, Laranja, F. S. and Nobrega, G. 1945, Doença de Chagas, *Mem. Inst. Oswaldo Cruz* **43:** 495–582.
13. Eakin, R. M. 1949, The nature of the organizer, *Science* **109:** 195–197.
14. Elkeles, G. 1944, Investigaciones sobre la evolución de *Trypanosoma cruzi*, especialmente sobre la génesis de la forma tripanosómica, *Bol. Acad. Nac. Cien.* **36:** 330–408.
15. ——— 1945, Experimental studies and critical considerations regarding the life cycle of *Trypanosoma cruzi*, *Am. J. Trop. Med.* **25:** 141–143.
16. ——— 1951, On the life cycle of *Trypanosoma cruzi*, *J. Parasit.* **37:** 379–386.
17. Fairbairn, H. and Culwick, A. T. 1946, A new approach to trypanosomiasis. With a statistical analysis by F. L. Gee, *Ann. Trop. Med. & Parasit.* **40:** 421–452.
18. Floch, H. and de Lajudie, P. 1941, Recherches sur la Trypanosomiase humaine américaine en Guyane Francaise. Les réservoirs de Virus, *Inst. Pasteur Guyane Terr. l'Inini*, No. 21, 1–6.
19. Goble, F. C. 1951, Studies on experimental Chagas' disease in mice in relation to chemotherapeutic testing, *J. Parasit.* **37:** 408–414.
20. Hartmann, M. 1910, Notiz über eine weitere Art der Schizogonie bei *Schizotrypanum cruzi* (Chagas), *Arch. Protist.* **20:** 361–363.
21. ——— 1917, Ueber die Schizogonie von *Schizotrypanum cruzi*, *Arch. Protist.* **38:** 113–116.
22. Hauschka, T. S. 1947, Sex of host as a factor in Chagas' disease, *J. Parasit.* **33:** 399–404.
23. ——— 1949, Persistence of strain-specific behavior in two strains of *Trypanosoma cruzi* after prolonged transfer through inbred mice, *J. Parasit.* **35:** 593–599.
24. Kirby, H. 1944, Some observations on cytology and morphogenesis in flagellate protozoa, *J. Morph.* **75:** 361–406.
25. Manso Soto, A. E. 1948, Enfermedad de Chagas, su diffusión en nuestro país, *Misión Estud. Patol. Reg. Argent.* **19:** 7–12.

26. Mayer, M. and da Rocha-Lima, H. 1912, Zur Entwicklung von *Schizotrypanum cruzi* in Saugetieren, *Arch. Schiffs- u. Tropen-Hyg.* **16:** 90–94.
27. ——— 1914, Zum Verhalten von *Schizotrypanum cruzi* in Warmblütern und Arthropoden, *Arch. Schiffs- u. Tropen-Hyg.* **18:** 101–136.
28. Mazza, S. 1940, Investigaciones sobre enfermedad de Chagas. IX. Otros mamíferos infectados naturalmente por *Schizotrypanum cruzi*, o *cruzi* similes en provincias de Jujuy y Salta, *Misión Estud. Patol. Reg. Argent.*, Publ. No. 45, 119–134.
29. ——— and Benitez, C. 1937. Comprobación de la naturaleza esquizotripanósica y frecuencia de la dacrioadenitis en la enfermedad de Chagas. Presencia de granulaciones tarsales en la forma ocular de esta enfermedad, *Misión Estud. Patol. Reg. Argent.*, Publ. No. 31, 3–31.
30. ——— and Driollet, E. 1935, Comprobación de otra especie de armadillo en San Juan con infección natural por *Schizotrypanum cruzi*, *Misión Estud. Patol. Reg. Argent.*, Publ. No. 25, 3–11.
31. ——— and Jörg, M. E. 1939, Anatomía patológica de la enfermedad de Chagas y de animales naturalmente infectados por "*Schizotrypanum cruzi*", *Actas y Trab. 6. Cong. Nac. Med.* (Cordoba) **3:** 3–29.
32. ——— and Romaña, C. 1934, Otro caso de forma aguda de enfermedad de Chagas en el norte Santafecino, *Misión Estud. Patol. Reg. Argent.*, Publ. No. 15, 25–34.
33. ——— Miyara, S. and Sanjurjo, H. E. 1936, Comprobación de animales domésticos y nuevos especies de mamíferos silvestres portadores de *Schizotrypanum cruzi* en los alrededores de la ciudad de Mendoza, *9. Reunión Soc. Argent. Patol. Reg.* (Mendoza) **1:** 548–559.
34. Mazzotti, L. 1940, Effects of inoculating small and large numbers of *Trypanosoma cruzi* into mice, *Am. J. Hyg.* (Sect. C) **31:** 86–91.
35. Nägler, K. 1913, Experimentelle Studien über die Passage von *Schizotrypanum cruzi* Chagas durch einheimische Tiere, *Centralbl. Bakt.* (Orig.) **71:** 202–206.
36. Niño, F. L. 1927, Schizotrypanosis experimentál en el gato, *Rev. Univ. Buenos Aires* **5:** 888–898.
37. ——— 1928, Aspectos morfológicos del "*Schizotrypanum cruzi*" en los organismos de sus huéspedes y en los medios de cultivo, *Bol. Inst. Clín. Quir.* **4:** 600–604.
38. Packchanian, A. 1939, Natural infection of *Triatoma gerstakeri* [sic] with *Trypanosoma cruzi* in Texas, *Publ. Health Repts.* **54:** 1547–1554.
39. Pappenheim, A. and Hirschfeld, H. 1920, *Haematologische Bestimmungstafeln*, Leipzig, Klinkhardt. 1–335.
40. Pessôa, S. B., Coutinho, J. O. and Moreira, J. D. 1941, Sôbre um caso de moléstia de Chagas (forma aguda), em Pedregulho, Estado de São Paulo, Brasil, *Rev. Clin. S. Paulo* **10:** 1–3.
41. Reina Guerra, A. 1939, Contribución al estudio de la Tripanosomiasis en el Salvador, Published by the author. 1–18.
42. Rey Matiz, H. 1941, Observaciones sobre trypanosomas en Colombia, *Rev. Fac. Med. Univ. Nac.* **10:** 25–49.
43. Robertson, A. 1929, Note on a trypanosome morphologically similar to *Trypanosoma cruzi* Chagas, 1909, found in an opossum, *Didelphis marsupialis*, captured at Tela, Honduras, Central America, *18 An. Rept. Med. Dept.* (United Fruit Co.), 294–310.
44. Vianna, G. 1911, Beitrag zum Studium der pathologischen Anatomie der Krankheit von Carlos Chagas. (Schizotrypanose des Menschen oder parasitaere Thyreoiditis), *Mem. Inst. Oswaldo Cruz* **3:** 276–294.
45. Wenyon, C. M. 1926, *Protozoology*. A manual for medical men, veterinarians and zoologists. In 2 volumes, London, Bailliere, Tindall and Cox. 1–1563.
46. Wood, F. D. 1934, Natural and experimental infection of *Triatoma protracta* Uhler and mammals in California with American human trypanosomiasis, *Am. J. Trop. Med.* **14:** 497–517.

47. Wood, S. F. 1949a, Additional observations on *Trypanosoma cruzi* Chagas from Arizona in insects, rodents, and experimentally infected animals, *Am. J. Trop. Med.* **29:** 43–55.
48. ——— 1949b, The occurrence of binary fission in the metacyclic form of *Trypanosoma cruzi* Chagas from *Triatoma longipes* Barber, *Bull. So. Calif. Acad. Sci.* **48:** 45–46.
49. ——— 1951a, Development of Arizona *Trypanosoma cruzi* in mouse muscle, *Am. J. Trop. Med.* **31:** 1–11.
50. ——— 1951b, The fragility factor for *Trypanosoma cruzi* in experimental Chagas' disease, *Bull. So. Calif. Acad. Sci.* **50:** 164–166.

Explanation of Plate 1

Intramuscular development of Brazilian *Trypanosoma cruzi*. All figures were drawn with the aid of a camera lucida from slides stained with Jenner-Giemsa. Figures 1 through 13, 15 and 16 are from gastrocnemius-muscle contact preparations of experiments 164 (Figures 1–3, 5–7, 9–13, 15 and 16) and 169 (Figures 4 and 8). Figure 17 is from a heat-dried slide of feces from an experimentally infected *Triatoma protracta*. Figures 14, 18 and 19 are from a heat-dried slide of tail blood of experiment 164 on the 14th day after inoculation. Experimental animal 164 was sacrificed on the 19th day and 169 on the 28th day. The developmental cycle is illustrated by Figures 1 through 14, exemplifying indirect development (Figures 9, 10, 11, and 12) of progressive parasites. Less frequently, direct development was observed (Figures 9, 15, 16, and 12).

Description of Figures

Figs. 1 and 2. Regressive trypanosomes.

Figs. 3 and 4. Regressive transition parasites.

Fig. 5. Regressive leishmaniform parasite.

Figs. 6 and 7. Dividing leishmaniform parasites.

Fig. 8. Progressive leishmaniform parasite.

Figs. 9 and 10. Progressive transition parasites.

Fig. 11. Early intermediate size, progressive trypanosome, after formation of V-shaped indentation and separation of extremities.

Figs. 12 and 13. Intermediate size, regressive blood and tissue trypanosomes.

Fig. 14. Intermediate size, regressive blood trypanosome.

Figs. 15 and 16. Developing trypanosomes by direct development or simple elongation.

Fig. 17. Metacyclic NIH Brazilian *Trypanosoma cruzi* from *Triatoma protracta*.

Fig. 18. Short, slender, progressive blood trypanosome.

Fig. 19. Long, slender, progressive blood trypanosome with extended posterior protoplasmic cap.

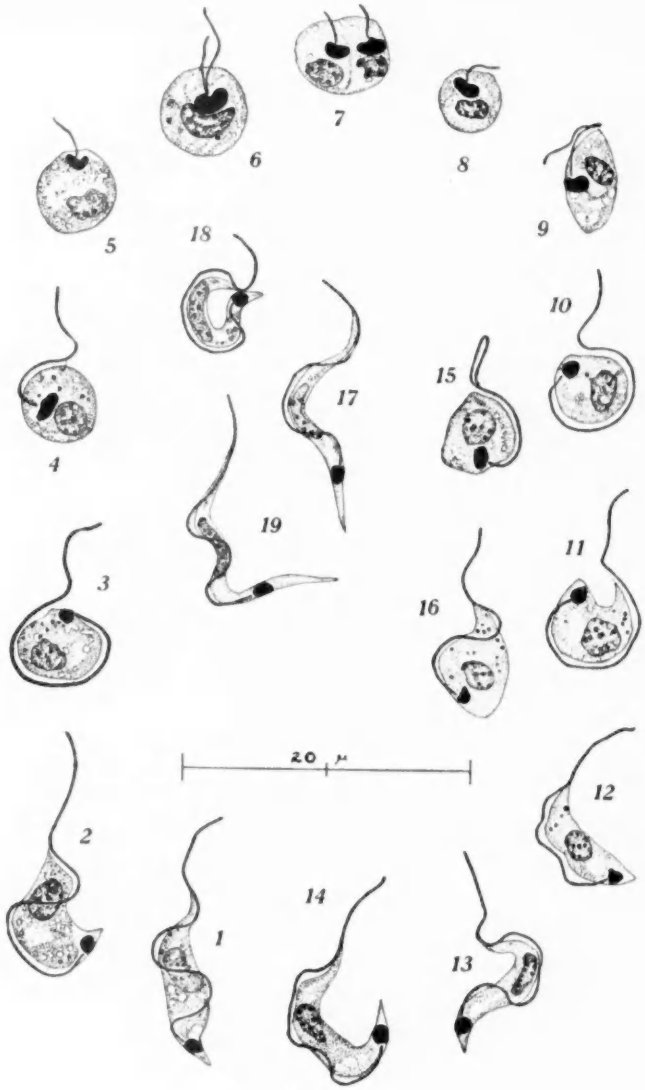

PLATE 1

THE CHEMOTHERAPEUTIC EFFICACY OF 2,4-DIAMINO-5-P-CHLOROPHENYL-6-ETHYLPYRIMIDINE (DARAPRIM) IN EXPERIMENTAL TOXOPLASMOSIS

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In recent investigations Eyles and Coleman (1952) demonstrated that 2,4-diamino-5-p-chlorophenyl-6-ethylpyrimidine (Daraprim) prevented death of white mice experimentally infected with a strain of *Toxoplasma gondii* isolated from the Norway rat. Additional studies by Eyles (1953) showed that the antitoxoplasmal action of Daraprim was enhanced by sulfadiazine.

In experimental toxoplasmosis it is well known that chemotherapy with such agents as sulfonamides, sulfones and certain antibiotics may prevent death of mice during the period of drug administration. It has been repeatedly observed however that *Toxoplasma* may not be eradicated from the tissues of mice and that relapse and death may occur within a variable time following cessation of therapy. It has been observed here that treated mice may survive for several months or possibly for the duration of their normal life span yet retaining foci of virulent *Toxoplasma* in their tissues. It is questionable therefore whether survival of *Toxoplasma*-infected mice during the period of experimental chemotherapy constitutes a valid criterion of drug efficacy. In view of these facts it has become increasingly important to observe treated animals for long periods of time after chemotherapy has been stopped to determine whether the organism has been eradicated from the tissues.

The experimental results presented here constitute an effort to evaluate Daraprim[2] in the chemotherapy of experimental toxoplasmosis. Successful chemotherapy of this disease in white mice does not necessarily portend similar success in treatment of human toxoplasmosis. Evaluation of Daraprim in human toxoplasmosis will undoubtedly be forthcoming.

MATERIALS AND METHODS

The strain of *Toxoplasma gondii* used in these studies was that isolated from a human infection by Sabin (1941) and is known as the R.H. strain. It is virulent for mice, rabbits and young rats but inoculation into old rats frequently results in a latent infection.

The organism in this work has been maintained by mouse passage of saline-diluted, peritoneal exudate every five days using 0.1 ml. of a 1–10 dilution injected intraperitoneally into 18–20 gram mice. Such animals usually died on the fifth day of infection, their peritoneal exudate containing 35–40 million free toxoplasma organisms per ml.

[1] This investigation was supported by a research grant (E-76C-3) from the Division of Research Grants of the National Institutes of Health, Public Health Service.

[2] The Daraprim used in these experiments was obtained through the courtesy of Wellcome Research Laboratories, Tuckahoe, New York.

Inoculation of animals used in the experimental studies was carried out in a similar but more extensive manner. Peritoneal exudate obtained on the fifth day of infection was diluted 1–10 in sterile saline. Additional serial dilutions of 1–100, 1–1000 and 1–10,000 were made. Experimental mice were inoculated intraperitoneally with 0.1 ml. of the 1–10 dilution. This procedure was repeated for each serial dilution thus creating four groups of six mice each, representing the four dilutions of the peritoneal exudate. Inoculated mice were housed in wire suspension-type cages in an air-conditioned animal room.

Administration of the test compounds was carried out by placing drugs in the animal food. A purified casein-sucrose ration supplemented with necessary growth factors and minerals was used as a basic animal food (Summers, 1947). This was provided daily in amounts that would insure four grams of food per mouse per day. Experimental feeding was begun either before or at the time of parasite inoculation. Water was provided ad libitum.

Evaluation of the effectiveness of the test compounds in preventing the death of inoculated animals during the period of drug administration was made by calculating the average time of survival in days and by determining the L.D. 50 titer in these mice according to the methods of Reed and Muench (1938). Evaluation of the effectiveness of drug treatment as a means of eradicating the parasite from the tissues of the treated animals was carried out by two methods. In the first, which applied only to those individuals which died spontaneously during and after treatment, peritoneal washings were examined microscopically for *Toxoplasma*. The material was obtained by injection of 1.0 ml. of sterile saline into the peritoneal cavity and withdrawing a small amount of fluid for microscopic examination. The tissues of treated animals that died spontaneously were not subinoculated into healthy mice.

The second method applied to treated animals that were sacrificed during the period of post-treatment observation. Here brain, spleen, liver and sometimes lung were transferred to healthy mice to determine the presence of viable *Toxoplasma*. Except for liver, in which case about one-half of the organ was used, entire organs were removed aseptically, ground lightly in a sterile mortar and diluted with 5 ml. of sterile saline. Two-tenth ml. aliquots of this tissue suspension were injected intraperitoneally into healthy mice. Thus for each treated, sacrificed animal from which brain, liver, spleen and lung were transferred there were twenty recipient mice or five for each tissue transferred. These mice were placed in clean suspension-type cages and fed on commercial animal ration, free of antibiotic supplement. Water was provided ad libitum.

EXPERIMENTAL RESULTS

Six multiple dilution studies involving the use of Daraprim as a chemotherapeutic agent against experimental toxoplasmosis are presented here. The results have been summarized in comprehensive tables.

Following the initial observation that Daraprim prevented the death of *Toxoplasma*-infected white mice, further studies were carried out to determine the extent to which the diet concentration of this drug could be reduced and

still produce beneficial effects. In early experiments (unpublished results) Daraprim was toxic to mice when fed at diet levels of 0.1 and 0.15 per cent. Toxicity was manifested by marked weight loss, ruffled coat and inactivity. Reduction of drug concentration was accompanied by lowered effectiveness in protection against the infection and fewer deaths among toxicity controls. Table 1 presents the results of two typical experiments in which Daraprim was

TABLE 1

Effect of 2,4-diamino-5-p-chlorophenyl-6-ethylpyrimidine on Toxoplasma *infection in mice*

TREATMENT	PROPORTION OF MICE DYING AT TOXOPLASMA DILUTIONS OF				LD_{50} TITER ON THE 8TH DAY	DEATH RATIO ON THE 12TH DAY	TOXICITY RATIO
	10^{-1}	10^{-2}	10^{-3}	10^{-4}			
1. Daraprim 0.01%	6/6	4/6	1/6	1/6	2.47	24/24	1/6
Control	6/6	6/6	3/6	3/6	3.85	24/24	—
2. Daraprim 0.01%	6/6	6/6	6/6	3/6	4.0	24/24	0/6
Control	6/6	6/6	6/6	3/6	4.0	24/24	—

TABLE 2

Effect of 2,4-diamino-5-p-chlorophenyl-6-ethylpyrimidine (Daraprim) on Toxoplasma gondii *fection in mice. In Part A of this experiment 10^{-1} animals received approximately 380,000 free* Toxoplasma *organisms*

A. RESULTS DURING TREATMENT PERIOD						B. EVALUATION OF CHEMOTHERAPY*						
Treatment	Proportion of mice dying at *Toxoplasma* dilutions of				LD_{50} titer on 9th day	Original dilution	Animal number					
	10^{-1}	10^{-2}	10^{-3}	10^{-4}			1	2	3	4	5	6
Daraprim 0.075%	2/6	0/6	0/6	0/6	−0.5	10^{-1}	6D−	8D−	10D−	11D−	22D+	23S+
						10^{-2}	10D−	11D−	15D+	23S−	41D−	84S−
						10^{-3}	11D−	12D−	79D−	79D−	84S−	84S−
						10^{-4}	198S−	198S−	198S−	198S−	198S−	198S−
Control	6/6	6/6	5/5	2/6	3.59	All control animals contained *Toxoplasma* parasites intraperitoneally at the time of death. All were dead by the end of the 11th day						

* Explanation of Table figures (Part B). D = animal died spontaneously and was examined for presence of *Toxoplasma* by peritoneal washing. S = animal was sacrificed and tissues were subinoculated into healthy animals. *Minus sign* following D or S indicates that *Toxoplasma* was not demonstrated in treated animal. *Plus sign* following D or S indicates that *Toxoplasma* was demonstrated in treated animal. *Numeral preceding* D or S indicates the number of days since administration of infective dose.

fed at 0.01 per cent concentration. In one experiment Daraprim was fed for 14 days, 7 days prior to inoculation and 7 days following inoculation. Animals representing the 1–10 dilution received approximately 420,000 free *Toxoplasma* parasites. In the second experiment Daraprim administration was begun at the time of inoculation and was continued for a period of nine days. The 1–10 dilution animals received approximately 390,000 free *Toxoplasma* parasites. In each experiment six uninoculated mice served as drug toxicity controls.

Toxoplasma were recovered from the peritoneal fluid of all Daraprim-treated animals beginning the fifth day of infection although the numbers of parasites were not as great as in the controls. Controls were positive for organisms beginning on the fourth day. It can be seen that the rate of death of Daraprim-treated animals was slightly less in experiment 1 above in which feeding was begun seven days prior to inoculation.

TABLE 3

Effect of 2,4-diamino-5-p-chlorophenyl-6-ethylpyrimidine (Daraprim) alone and in combination with pteroylglutamic acid, on Toxoplasma gondii *infection in mice. In Part A of this experiment 10^{-1} animals received approximately 380,000 free* Toxoplasma *organisms*

A. RESULTS DURING TREATMENT PERIOD						B. EVALUATION OF CHEMOTHERAPY*						
Treatment	Proportion of mice dying at *Toxoplasma* dilutions of				LD_{50} titer on 9th day	Original dilution	Animal number					
	10^{-1}	10^{-2}	10^{-3}	10^{-4}			1	2	3	4	5	6
Daraprim 0.1%	0/6	0/6	1/6	1/6	−0.5	10^{-1}	10D−	18D+	41S−	57S−	57S−	57S−
						10^{-2}	13D−	13D−	19D+	85S−	85S−	85S−
						10^{-3}	5D−	10D−	12D−	30D−	54D−	84S−
						10^{-4}	9D−	11D−	11D−	29D−	30D−	84S−
Daraprim 0.1% plus PGA 0.05%	0/6	1/6	0/6	0/6	−0.5	10^{-1}	17D+	17D+	17D+	41S−	57S−	57S−
						10^{-2}	5D−	17D+	29D−	92S−	92S−	92S−
						10^{-3}	10D−	52D−	92S−	92S−	92S−	92S−
						10^{-4}	12D−	18D−	92S−	92S−	92S−	92S−
PGA 0.05%	6/6	6/6	6/6	3/3	4.0	All PGA-fed and control animals contained *Toxoplasma* intraperitoneally at the time of death. All were dead by the end of the 12th day						
Control	6/6	6/6	6/6	3/6	4.0							

* Explanation of Table figures. (Part B). D = animal died spontaneously and was examined for presence of *Toxoplasma* by peritoneal washing. S = animal was sacrificed and tissues were subinoculated into healthy animals. *Minus sign* following D or S indicates that *Toxoplasma* was not demonstrated in treated animal. *Plus sign* following D or S indicates that *Toxoplasma* was demonstrated in treated animal. *Numeral preceding* D or S indicates the number of days since administration of infective dose.

It was obvious from the results presented above that higher drug levels of Daraprim would be required to produce maximum protection and possible cure of *Toxoplasma*-infected mice. In the four experiments described in Tables 2, 3, 4 and 5 Daraprim was fed at 0.075 or 0.1 per cent in the ration. It was known that such drug levels caused the death of a few animals and that all lost considerable weight during the period of chemotherapy. In all of the four experiments Daraprim was fed for a period of nine days beginning on the day of inoculation. Animals that died during the course of treatment and afterwards were examined for *Toxoplasma* according to the methods described previously. Survivors were sacrificed at varying intervals during the experiment. Brain, spleen, liver and lung were subinoculated into healthy mice.

Because of results obtained in earlier studies (Summers, 1947) with sulfonamides and sulfones an additional series of mice was included in these experi-

ments in order to test the effects of pteroylglutamic acid (PGA) on the action of Daraprim.

DISCUSSION OF RESULTS

The results of these experiments confirm in general the observations in previous investigations, namely, that 2,4-diamino-5-p-chlorophenyl-6-ethylpyrimidine (Daraprim), when fed at sufficiently high concentration may inhibit the growth of *Toxoplasma gondii* in experimentally infected white mice. Retardation or prevention of parasite growth and prolongation of host life has been interpreted as the specific effect of administration of the drug.

The inhibition of parasite growth and prolongation of host survival has not been accepted here as the sole measure of chemotherapeutic efficacy of the drug. In experimental toxoplasmosis, relapse and death may follow drug therapy, and in many animals latent infection may occur with virulent parasites residing in various tissues of the drug-treated host. The true measure of chemotherapeutic efficacy in this infection must include, as has been done here, observations to determine the presence of post-treatment relapse and latent infection.

At low drug levels Daraprim (.01 per cent) neither prevented development of the parasite nor death of the animals. In four additional experiments in which Daraprim was fed at levels of 0.075 or 0.1 per cent many animals survived the period of drug administration, but the drug showed a high degree of toxicity in the uninfected mice. Furthermore high levels of Daraprim did not always eradicate the parasite from the tissues of the mice. Incorporation of pteroylglutamic acid in the Daraprim diet resulted in fewer deaths and less weight loss in uninfected animals. *Toxoplasma*-infected animals that received the combination diet seemed to fare better during the period of treatment and thereafter. These findings may indicate that PGA reduced the toxicity of Daraprim for the white mouse. There was little evidence that PGA interfered with the antitoxoplasmal activity of Daraprim.

In experiments in which Daraprim was fed by itself at 0.075 or 0.1 per cent, twenty or approximately 21 per cent of animals died during the period of treatment. The percentage that died was higher among those that received 0.1 per cent Daraprim. When PGA was included in the diet either at 0.05 or 0.1 per cent along with 0.1 per cent Daraprim only four or 5.5 per cent of the animals died during the period of drug administration. Drug toxicity studies on parasite free mice support these findings. *Toxoplasma* were never observed in the peritoneal washings of animals fed Daraprim at 0.075 or 0.1 per cent or Daraprim-PGA diet during the period of drug administration.

In all experiments a second period of observation comprised the time from the 10th day through the 23rd day post-infection. Previous experience indicated this to be the most critical period with regard to relapse and death of treated animals. Twenty per cent of animals that received Daraprim alone at 0.075 or 0.1 per cent died during this period. Five or twenty-six per cent were found to contain *Toxoplasma* by the peritoneal washing method. Among the groups that received Daraprim-PGA diet eleven or 15 per cent died during the 10–23 day period and

five or 45 per cent contained *Toxoplasma* in the peritoneal cavity as demonstrated by examination of peritoneal washings.

Ten animals that were fed Daraprim alone at 0.075 or 0.1 per cent were sacrificed during the 10–23 day period. Thirty per cent were found to contain *Toxo-plasma* by tissue subinoculation. Eight Daraprim-PGA fed animals were sacrificed during this period of observation and one or 12.5 per cent were found infected by tissue subinoculation.

TABLE 4

Effect of 2,4-diamino-5-p-chlorophenyl-6-ethylpyrimidine (Daraprim) alone and in combination with pteroylglutamic acid on Toxoplasma gondii *infection in mice. In Part A of this experiment 10^{-1} animals received approximately 410,000 free* Toxoplasma *organisms*

A. RESULTS DURING TREATMENT PERIOD						B. EVALUATION OF CHEMOTHERAPY*						
Treatment	Proportion of mice dying at *Toxoplasma* dilutions of				LD_{50} titer on 9th day	Original dilution	Animal number					
	10^{-1}	10^{-2}	10^{-3}	10^{-4}			1	2	3	4	5	6
Daraprim 0.1%	3/6	1/6	1/6	4/6	1.75	10^{-1}	3D−	6D−	9D−	10D−	10D−	45S−
						10^{-2}	9D−	16D−	21D−	24D−	45S−	45S−
						10^{-3}	9D−	9D−	45S−	45S−	45S−	45S−
						10^{-4}	6D−	6D−	7D−	9D−	10D−	45S−
Daraprim 0.1% plus PGA 0.1%	2/6	0/6	0/6	0/6	−0.5	10^{-1}	1D−	2D−	43D−	46S−	46S−	46S−
						10^{-2}	40D−	46S−	46S−	46S−	46S−	46S−
						10^{-3}	46S−	46S−	46S−	46S−	46S−	46S−
						10^{-4}	43D−	47S−	47S−	47S−	47S−	47S−
PGA 0.1%	6/6	6/6	6/6	5/6	4.0+	All PGA-fed and control animals contained *Toxoplasma* intraperitoneally at the time of death. All were dead at the end of the 10th day						
Control	6/6	6/6	6/6	5/6	4.0+							

* Explanation of Table figures. (Part B). D = animal died spontaneously and was examined for presence of *Toxoplasma* by peritoneal washing. S = animal was sacrificed and tissues were subinoculated into healthy animals. *Minus sign* following D or S indicates that *Toxoplasma* was not demonstrated in treated animal. *Plus sign* following D or S indicates that *Toxoplasma* was demonstrated in treated animal. *Numeral preceding* D or S indicates the number of days since administration of infective dose.

A third period of observation included that period from the 23rd post-infection day to the termination of observation. Thirty-two or 33 per cent of all animals which received Daraprim alone at 0.075 or 0.1 per cent were sacrificed for tissue subinoculation during the third period. In some experiments this period of observation lasted as long as 198 days post-infection and in others as little as 29 days post-infection. This variation in time of sacrifice of treated survivors was purposely carried out in order to detect any post-treatment time during which the parasite might become reactivated following treatment with Daraprim. It was not possible to produce infection in healthy recipient mice by subinoculation of tissues from animals in this group that survived more than 23 days. None of the animals that died during this period were found to harbor *Toxoplasma* in the peritoneal cavity.

Forty-two Daraprim-PGA-treated animals were sacrificed at various intervals

during the third period of observation. Likewise none of these were found to harbor virulent *Toxoplasma* by tissue subinoculation. None of those that died during this terminal period of the experiments contained *Toxoplasma* in peritoneal washings. It is significant that *Toxoplasma* could not be demonstrated in peritoneal washings during treatment with Daraprim or Daraprim-PGA; that they were demonstrable, however, as early as three days post-treatment and as late as the 14th day post-treatment and finally that parasites were never demonstrable beyond the 14th post-treatment day.

TABLE 5

Effect of 2,4-diamino-5-p-chlorophenyl-6-ethylpyrimidine (Daraprim) alone and in combination with pteroylglutamic acid, on Toxoplasma gondii *infection in mice. In part A of this experiment 10^{-1} animals received approximately 360,000 free* Toxoplasma *organisms*

A. RESULTS DURING TREATMENT PERIOD						B. EVALUATION OF CHEMOTHERAPY*						
Treatment	Proportion of mice dying at *Toxoplasma* dilutions of				LD_{50} titer on 9th day	Original dilution	Animal number					
	10^{-1}	10^{-2}	10^{-3}	10^{-4}			1	2	3	4	5	6
Daraprim 0.1%	1/6	2/6	2/6	1/6	1.21	10^{-1}	6D−	10D−	12S+	14D−	17S−	18D+
						10^{-2}	8D−	8D−	12S−	17S−	24D−	29S−
						10^{-3}	5D−	9D−	12S−	17S+	29S−	29S−
						10^{-4}	9D−	12S−	17S−	29S−	29S−	29S−
Daraprim 0.1% plus PGA 0.1%	0/6	0/6	1/6	0/6	−0.5	10^{-1}	12S−	16D+	16D−	17S+	19D−	29S−
						10^{-2}	12S−	17S−	29S−	29S−	29S−	29S−
						10^{-3}	7D−	12S−	17S−	15D−	26D−	26D−
						10^{-4}	12S−	17S−	29S−	29S−	29S−	29S−
PGA 0.1%	6/6	6/6	6/6	5/6	4.0−	All PGA-fed and control animals contained *Toxoplasma* intraperitoneally at the time of death. All were dead at the end of the 11th day						
Control	6/6	6/6	5/6	4/6	4.0−							

* Explanation of Table, figures. (Part B). D = animal died spontaneously and was examined for presence of *Toxoplasma* by peritoneal washing. S = animal was sacrificed and tissues were subinoculated into clean animals. *Minus sign* following D or S indicates that *Toxoplasma* was not demonstrated in treated animal. *Plus sign* following D or S indicates that *Toxoplasma* was demonstrated in treated animal. *Numeral preceding* D or S indicates the number of days since administration of infective dose.

Examination of Tables 2, 3, 4, and 5 will reveal that the numbers of parasites inoculated into experimental animals were comparable in each experiment. None of the animals that received the 1–10,000 strength inoculum were found to harbor *Toxoplasma* during or after treatment. This applies to both Daraprim and Daraprim-PGA-treated animals. All untreated control animals inoculated with this dilution of the parasite died and all were heavily infected. It is concluded that Daraprim was responsible for eradication of the parasite from animals when the inoculum contained only a few organisms. In one instance *Toxoplasma* was demonstrated by tissue subinoculation in an animal that had received the 1–1000 strength inoculum. Here again untreated controls died and all were heavily infected.

Three Daraprim or Daraprim-PGA-treated animals representing the 1–100 dilution of inoculum were found to harbor *Toxoplasma* following treatment. Infection was detected both by peritoneal washing and tissue subinoculation methods.

Ten animals that received the 1–10 dilution of inoculum were found to be infected during the post-treatment period. The majority of these infections were detected by the peritoneal washing method.

It seems clear therefore, that the interpretation placed on chemotherapeutic effectiveness of a given chemical compound in experimental toxoplasmosis must take into account the number of parasites against which the drug has to act and the toxic effects of the drug on the host. Clearly, toxic dosages of Daraprim will eradicate *Toxoplasma* from mice, when given at the start of the infection.

CONCLUSIONS

Low diet concentrations of Daraprim (0.01 per cent) did not prevent the death of *Toxoplasma*-infected mice nor alter the development of the parasite in the host. Higher, but clearly toxic dosages of Daraprim (0.075 and 0.1 per cent) prevented the development of *Toxoplasma gondii* and protected a large percentage of infected white mice. The addition of pteroylglutamic acid to the Daraprim diet did not reverse the anti-toxoplasmal action of Daraprim and appeared to lessen the toxic action of the latter for the animals. Judging by the methods used, Daraprim did consistently eradicate *Toxoplasma* from brain, spleen, liver and lungs of mice that received small to moderately heavy inocula of this parasite.

REFERENCES

EYLES, DON E., AND COLEMAN, NELL, 1952. Tests of 2, 4-diaminopyrimidines on toxoplasmosis, *Public Health Reports* **67:** 249–252.

EYLES, DON E., 1953. The present status of the chemotherapy of toxoplasmosis, in press.

SABIN, A. B., 1941. Toxoplasmic encephalitis in children, *J.A.M.A.* **116:** 801–807.

SUMMERS, WILLIAM A., 1947, Antagonism of sulfonamide inhibition by para-aminobenzoic acid and folic acid in *Toxoplasma* infected mice, *Soc. Exp. Biol. and Med.* **66:** 509–511.

THE *IN VITRO* EFFECT OF VARIOUS ANTIBIOTICS ON *TRICHOMONAS VAGINALIS*

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Tyrothricin and tyrocidin were found by Trussel (1947) to be effective against *Trichomonas vaginalis* in useful concentrations in acidified serum, while gramicidin, streptomycin, hemopyocyanine and sodium penicillin were not effective. McVay *et al.* (1949, 1951) established that aureomycin was trichomonicidal *in vitro*, but reported that the administration of two grams of aureomycin a day by mouth for seven days had no destructive effect on *Trichomonas vaginalis*. They treated 54 patients by insufflation of 500 mg. of aureomycin on the first, second, third, fourth, sixth and eighth days, and subsequently inserted 250 mg. capsules of aureomycin into the vagina nightly for two weeks. The patients were followed for a period of two to three months. Six had relapse or reinfection, but were subsequently successfully treated. Blood levels of aureomycin three hours after vaginal insufflation were in the neighborhood of 0.12 μg./ml. Twelve of these patients were followed for six months and in two, trichomonads reappeared. In another investigation McVay *et al.* (1950) treated 95 patients having *Trichomonas* vaginitis by the local application of aureomycin. Sixty-three were nonpregnant and thirity-two were pregnant. Eighty-eight were colored and seven were white. Eighty-six patients were cured according to their criteria. The treatment included insufflation of aureomycin and then the insertion of 250 mg. aureomycin gelatin capsules into the vagina every other night. The entire treatment lasted two weeks.

Greenblatt and West (1949, 1950) studied the effect of streptomycin, aureomycin, tyrothricin, penicillin and chloramphenicol on *Trichomonas vaginalis*. The trichomonads were obtained from the vagina of patients and were suspended in various concentrations of the antibiotics. They concluded that penicillin and streptomycin had minimal effect on *Trichomonas vaginalis* while tyrothricin and aureomycin were fairly effective. Greenblatt and Barfield (1951) used aureomycin to treat fifty-three patients suffering from *Trichomonas* vaginitis. In forty-eight patients the vaginal secretions were examined and were positive for *T. vaginalis*. Forty-seven of the forty-eight cases were cured. In one patient the organisms persisted during the course of therapy. In 78.3 per cent of the patients there was no recurrence within one month. Vaginitis due to a yeastlike organism occurred in 15.1 per cent of the patients.

Patiala and Vara (1951) in Finland treated 77 cases of *Trichomonas* vaginitis with aureomycin and got 67.5 per cent recovery; 40 cases were treated with terramycin with 72.5 per cent recovery. The minimum follow-up period was three weeks.

Green (1952) treated twenty-one patients suffering from *Trichomonas* vaginitis with 100 mg. terramycin vaginal suppositories for a period of one week.

Fourteen patients were followed for a period of six months and found to be cured. There was only one recurrence which responded to a ten day course of terramycin treatment.

Feo (1952) treated nine pregnant patients suffering from *Trichomonas* vaginitis with 600,000 units of penicillin daily for ten days with negative results. He also treated ten patients with dihydrostreptomycin, one gram a day, from three to forty days, alone or with para-amino salicylic acid, and could not demonstrate any diminution of trichomonads in the vagina. Five other patients were given 250 or 500 mg. of terramycin every six hours for seven to fourteen days; six patients received 250 or 500 mg. of aureomycin every three to six hours for three to twenty-two days; and eight other patients were given 250 to 500 mg. of chloramphenicol every six hours for five to twenty-one days. All cases failed to respond to treatment and there was no effect on the trichomonads of the vagina even though a favorable influence on the abnormal vaginal flora was apparent.

TABLE 1

The effect of Polymyxin, terramycin, bacitracin, carbomycin (Magnamycin), Neomycin and Viomycin on cultures of T. vaginalis

CONCENTRATION OF ANTIBIOTIC	POLYMYXIN	POLYMYXIN-TERRAMYCIN	TERRAMYCIN	BACITRACIN	CARBOMYCIN	NEOMYCIN	VIOMYCIN
1:1,000	−	−	−	+	−	−	−
1:2,000	−	−	−	+	−	−	+
1:4,000	−	−	−	+	+	−	+
1:8,000	−	+	+	+	+	−	+
1:16,000	+	+	+	+	+	+	+
1:32,000	+	+	+	+	+	+	+
1:64,000	+	+	+	+	+	+	+
1:128,000	+	+	+	+	+	+	+
1:256,000	+	+	+	+	+	+	+
Control	+	+	+	+	+	+	+

+ means growth. − means no growth.

The present investigations were undertaken to study the effect of antibiotics currently in use and especially the newer antibiotics on cultures of *Trichomonas vaginalis* and *Trichomonas foetus*.

MATERIAL AND METHODS

Trichomonas vaginalis and *Trichomonas foetus* were cultured in simplified trypticase serum medium (Kupferberg *et al.*, 1948) for 48 hours at 37°C. Since there was no significant difference between the two species, to avoid duplication only the results for *Trichomonas vaginalis* are listed. The antibiotics were dissolved serially in 3 ml. of the culture medium and about 100,000 actively motile trichomonads were inoculated into each tube. The tubes were incubated at 37°C. and readings were taken at twenty-four and forty-eight hour intervals. There was no difference in the two readings.

RESULTS

Table 1 shows that polymyxin inhibits *T. vaginalis* in 1:8,000 (125 μg./ml.). Terramycin and equal concentrations (by weight) of polymyxin-terramycin mixture inhibit *T. vaginalis* in 1:4,000 (250 μg./ml.). Bacitracin does not inhibit *T. vaginalis* in 1:1,000 concentration (1 mg./ml.), while carbomycin (Magnamycin) inhibits it in a concentration of 1:2,000 (500 μg./ml.). Neomycin inhibits

TABLE 2

The effect of Thiolutin, Deacetylthiolutin and Propionamido-deacetylthiolutin on cultures of T. vaginalis

CONCENTRATION OF ANTIBIOTIC	THIOLUTIN	DEACETYLTHIOLUTIN	PROPIONAMIDO-DEACETYLTHIOLUTIN
1:140,000	−	−	−
1:280,000	−	−	−
1:560,000	−	+	−
1:1,120,000	−	+	−
1:2,240,000	−	+	−
1:4,480,000	−	+	−
1:8,960,000	+	+	+
Control	+	+	+

+ means growth. − means no growth.

TABLE 3

The effect of Streptothricin, Netropsin, Rimocidin and Fumagillin on cultures of T. vaginalis

CONCENTRATION OF ANTIBIOTIC	STREPTOTHRICIN	NETROPSIN	RIMOCIDIN	FUMAGILLIN
1:10,000	−	−	−	−
1:20,000	−	−	−	−
1:40,000	−	−	−	−
1:80,000	+	−	+	+
1:160,000	+	+	+	+
1:320,000	+	+	+	+
Control	+	+	+	+

+ means growth. − means no growth.

T. vaginalis in a concentration of 1:8,000 (125 μg./ml.) and Viomycin inhibits in a concentration of 1:1,000 (1 mg./ml.).

Table 2 shows that Thiolutin and Propionamido-deacetylthiolutin inhibit *T. vaginalis* in a concentration of 1:4,480,000 (0.2 μg./ml.) and Deacetylthiolutin in a concentration of 1:280,000 (3.575 μg./ml.).

Table 3 shows that Rimocidin, fumagillin and streptothricin inhibit *T. vaginalis* in a concentration of 1:40,000 (25 μg./ml.), and Netropsin in a concentration of 1:80,000 (12½ μg./ml.).

DISCUSSION

An ideal trichomonicidal agent is one which is effective only against *Trichomonas vaginalis* but not on the normal bacterial flora of the vagina, whether the therapeutic agent is applied locally or given systemically. In the absence of normal vaginal flora, *Candida albicans* and *Staphylococcus* may overgrow unopposed and give rise to monilial vaginitis.

Thiolutin inhibits the growth of most bacteria in 1 to 10 μg./ml.; *Brucella bronchiseptica* and *Pseudomonas aeruginosa* are notable exceptions. It also inhibits pathogenic fungi in concentrations of 5 to 25 μg./ml., *Endamoeba histolytica* in 62½ to 125 μg./ml. (Seneca *et al.*, 1952), and the hemoflagellates in a concentration of 0.125 to 0.25 μg./ml. (Seneca).

Trichomonas vaginalis is inhibited by Thiolutin in a concentration of 0.2 μg./ml. It may prevent the overgrowth of *Candida albicans* and *Staphylococcus*. Deacetylthiolutin is effective in 3.575 μg./ml. and therefore it may conceivably be the second choice. Netropsin is effective in a concentration of 12½ μg./ml. and is possibly the third choice. Rimocidin, streptothricin and fumagillin are equally effective in a concentration of 25 μg./ml.

Terramycin, Neomycin, polymyxin and carbomycin (Magnamycin) have practically the same spectrum, being inhibitory in a concentration of 125 to 250 μg./ml., while bacitracin and Viomycin are ineffective. Carbomycin has a specific effect on Gram-positive and Gram-negative cocci in concentrations of 0.075 to 1.25 μg./ml. It is also effective against antibiotic resistant staphylococci and streptococci (English *et al.*; Tanner *et al.*). Broad spectrum antibiotics completely destroy the vaginal flora.

In the treatment of *Trichomonas vaginalis* care should be taken to prevent reinfection. This is particularly important in intercourse, because *T. vaginalis* occurs in the prostatic secretions and urine of male subjects.

The incidence of *Trichomonas* infections is high (Belding, 1952, Craig and Faust, 1951). Twenty-seven per cent of 978 nonpregnant women and 25 per cent of 1429 pregnant women were infected. In 314 pregnant colored women the rate of infection was 31 per cent, and 13 per cent in 286 pregnant white women. Among the patients who have venereal infections, 67 per cent were infected with *T. vaginalis*. Thirty per cent of the gynecological patients and 21 per cent of the obstetrical patients have been found to be infected. The incidence of infection increases when feminine hygiene is neglected. In women who have vaginitis the incidence of infection among 365 women was 54 per cent. Thirty seven per cent of 246 persons suffering from nonspecific urethritis were found infected with *T. vaginalis*. Among men the incidence is 9 per cent of 2,326 examinations. Among army recruits *T. vaginalis* was found in 16 per cent of 926 individuals.

SUMMARY AND CONCLUSION

In vitro culture studies indicate that Thiolutin and its Propionamido-deacetyl derivative inhibit *Trichomonas vaginalis* in a concentration of 0.2 μg./ml. Netropsin inhibits in 12½ μg./ml., and streptothricin, Rimocidin and fumagillin in a concentration of 25 μg./ml. Terramycin, Neomycin, polymyxin, carbomycin

(Magnamycin), polymyxin-terramycin mixture inhibit in a concentration of 125 to 250 μg./ml., while bacitracin and Viomycin are relatively ineffective.

REFERENCES

BELDING, D. L., 1952. *Clinical Parasitology*, 2nd Edition, Appleton-Century-Crofts, New York.

CRAIG, C. F., AND FAUST, E. C., 1951. *Clinical Parasitology*, 5th Edition, Lea and Febiger, Philadelphia.

ENGLISH, A., FIELD, M. F., SZENDY, S. R., TAGLIANI, N. J., AND FITTS, R. A., 1952. I. Magnamycin, in vitro studies, *Antibiotics and Chemotherapy* **2:** 678–688.

ENGLISH, A., MULLADY, H. E., AND FITTS, R. A., 1953. II. Magnamycin, in vivo studies, *Antibiotics and Chemotherapy* **3:** 94–98.

FEO, L. G., 1952. In vivo susceptibility of *Trichomonas vaginalis* to antibiotic therapy, *Am. J. Trop. Med. & Hyg.* **1:** 623–625.

GREENBLATT, R. B., AND BARFIELD, W. E., 1951. Aureomycin in the therapy of *Trichomonas vaginalis* vaginitis, *Am. J. Obst. & Gynec.* **62:** 423–425.

GREENBLATT, R. B., AND WEST, R. M., 1949. Antibiotics in treatment of *Trichomonas vaginalis*, *Journal of the Medical Association of Georgia* **38:** 350–352.

GREENBLATT, R. B., AND WEST, R. M., 1950. In vitro studies on the trichomonicidal effect of certain drugs and antibiotics, *Urological Cut. Review* **54:** 72–77.

GREENE, H. J., 1952. Terramycin in the treatment of *Trichomonas vaginalis* vaginitis, A report of 21 cases, *Antibiotics and Chemotherapy* **2:** 119–122.

KUPFERBERG, A. B., JOHNSON, G., AND SPRINCE, H., 1948. Nutritional requirements of *Trichomonas vaginalis*, *Proc. Soc. Exp. Biol. & Med.* **67:** 304–308.

MCVAY, L. V., EVANS, L., FLANAGAN, J. B., AND SPRUNT, D. H., 1949. Treatment of Trichomonas vaginitis with aureomycin, *Proc. Soc. Exper. Biol. & Med.* **72:** 674–675.

MCVAY, L. V., EVANS, L., AND SPRUNT, D. H., 1951. A new method of treatment of *Trichomonas vaginalis* vaginitis, *Surgery, Gynec. & Obst.* **93:** 177–184.

MCVAY, L. V., LAIRD, R. L., AND SPRUNT, D. H., 1950. The treatment of amebiasis with aureomycin, *South. Med. J.* **43:** 308–313.

PATIALA, R., AND VARA, P., 1951. Vaginitis produced by *Trichomonas vaginalis*, treatment with aureomycin and terramycin, *Ann. Med. Exper. Biol. Fenniae* **29:** 242–246.

SENECA, H. Unpublished data.

SENECA, H., KANE, J. AND ROCHENBACH, J., 1952. Bactericidal, protozoicidal and fungicidal properties of thiolutin, *Antibiotics and Chemotherapy* **2:** 1–4.

TANNER, F. W., ENGLISH, A. R., LEES, T. M., AND ROUTIEN, J. B., 1952. Some properties of Magnamycin, a new antibiotic, *Antibiotics and Chemotherapy* **2:** 241–243.

TRUSSEL, R. E., 1947. *Trichomonas vaginalis* and trichomoniasis. Charles C. Thomas, Springfield, Illinois, P. 61.

THE USE OF DIETHYLCARBAMAZINE (HETRAZAN OR NOTEZINE) IN TAHITI AS AN AID IN THE CONTROL OF FILARIASIS[1]

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In the development of the filariasis control program in French Oceania both drug control, by the use of diethylcarbamazine (Hetrazan, Notezine) and mosquito control have been employed. The earlier evaluation of the effect of diethylcarbamazine has been made in preliminary and progress reports from certain experimental areas and districts following either one year or two years of treatment, and was presented on the basis of (1) percentages of individuals positive for microfilariae before and after treatment and (2) the average number of microfilariae per 20 cmm. of blood before and after treatment. See Galliard and Mille (1949); Galliard, Mille and Robinson (1949); Beye, Edgar, Mille, Kessel and Bambridge (1952); Beye, Kessel, Bambridge, Thooris and Heuls (1952); Beye (1952).

Only preliminary data concerning the mosquito control program as applied to the first experimental areas (Beye *et al.*, 1952) have been published. It is considered that more extensive observations over longer periods of time should be made before generalizations concerning the comparative values of mosquito control procedures can be made.

MATERIALS AND METHODS

In this report the results of intensive treatment with diethylcarbamazine of 145 persons positive for microfilariae are compared with the general results of the routine field work. These 145 individuals were checked at the end of the first year after treatment and all positives were again treated; these were checked a year later and the results compared with those obtained in the following three different areas:

1) The combined districts of Faaone, Hitiaa, Mahaena, and Tiarei in which identical procedures of diethylcarbamazine administration, clean-up and application of DDT were used.
2) The district of Paea, where studies first began in this program, but which district has not been as well supervised as the districts listed under (1) above.
3) The island of Maiao where diethylcarbamazine alone, without mosquito control, was used.

Early in the program the question arose concerning the reduction of micro-

[1] Aided by contributions to the Pacific Tropic Diseases Research Project, School of Medicine, University of California, Los Angeles, by Cornelius Crane, Florence Belosselsky, William A. Robinson, Church of Jesus Christ of the Latter-Day Saints, Hofheimer Foundation, James P. McConnaughey, Grover C. Miller, Nicholas G. Rutger and anonymous friends.

filariae in man necessary to limit transmission to a nondangerous level or to eliminate it. In view of the fact that little or nothing was known concerning the minimum number of microfilariae in the blood stream of man necessary to institute infection in the mosquito vector, the following concepts presented themselves:

1) That mosquitoes feeding on people with low microfilarial rates probably acquire light or negligible infections.
2) That mosquitoes feeding on people with high microfilarial rates may acquire heavy infections but experience a high mortality rate resulting from parasitism.
3) That mosquitoes feeding on people with intermediate infections may be the most effective transmitters.

It was entirely possible that a marked decrease in the average microfilarial count in the general population might not prevent transmission of filarial infection under certain circumstances. Such transmission might continue to take place if mosquitoes could acquire sufficient microfilariae from persons with low counts and not be subjected to the mortality probably occasioned by feeding on a population with high counts.

Hoping to procure a quantitative answer to this problem, Dr. Leon Rosen of the Tropical Diseases Laboratory, U. S. Public Health Service,[2] was associated with the Filariasis Research Project in Tahiti in a study of the infection rates of mosquitoes when fed on human carriers harboring various levels of microfilariae per 20 cmm. of blood. Certain infection rates obtained by Rosen (1952) when feeding mosquitoes on human subjects harboring microfilariae at selected levels are quoted and an arbitrary infection factor obtained therefrom.

Evaluation of the differences in numbers of microfilariae in man before and after treatment with diethylcarbamazine would appear to have the greatest significance in estimating the progress or success of a control program if tabulated according to the frequency distribution of microfilariae per 20 cmm. of blood. Accordingly, the following levels were selected for comparison in this study: 1–5; 6–10; 11–30; 31–50; 51–100; and 100+, respectively. These are the same levels used in summarizing Rosen's experiments as shown in Table 5.

The dosage schedule of diethylcarbamazine used in most of this program was 2 mg. per kg. of body weight, given 3 times a day for 7 days. Several additional dosage schedules also were studied and among these 2 mg. per kg. of body weight, 3 times a day, 1 day each month for a year has afforded the greatest promise. The results obtained by this schedule are compared with the seven-day schedule.

RESULTS AND DISCUSSION

I. Treatment schedules

In most reports concerning the use of diethylcarbamazine the results have been based on a comparison of numbers or percentages of individuals positive before and after the administration of the drug. (See Hewitt *et al.* 1947, 1950,

[2] Our appreciation is expressed to Doctor Willard H. Wright, Director Tropical Diseases Laboratories, National Institutes of Health, Bethesda, Maryland, for his encouragement and friendly advice during the development of the work here reported.

1950a; Kenny and Hewitt, 1950; Oliver-Gonzalez and Santiago-Stevenson, 1949). The present authors are not aware of instances in which the reports have been made on as extensive a series and for as long a time as in the studies here recorded.

For comparison with previous results of other authors and of studies in Tahiti, the tables show (1) the numbers and percentages of individuals positive for microfilariae, (2) the average number of microfilariae per 20 cmm. of blood and (3) the number and percentage of individuals both before and after treatment occurring at each of the six levels selected for microfilarial counts.

TABLE 1

Results of treating 145 individuals, positive for microfilariae, with diethylcarbamazine given 3 times a day for one week each year

FREQUENCY DISTRIBUTION OF MICROFILARIAE PER 20 CM.	BEFORE TREATMENT		ONE YEAR AFTER FIRST TREATMENT		ONE YEAR AFTER SECOND TREATMENT	
	No.	%	No.	%	No.	%
0	0	0.0	87	60.0	120	82.5
1–5	31	21.4	33	23.0	20	14.0
6–10	15	10.4	11	7.5	3	2.0
11–30	33	22.7	11	7.5	2	1.5
31–50	23	15.9	2	1.4	0	0.0
51–100	10	6.9	0	0.0	0	0.0
100+	33	22.7	1	0.6	0	0.0
Total +	145	100	58	40	25	17.5
Average no. of microfilariae per 20 cmm.	79		3.4		0.8	

The percentages in all instances are based on the original 145 positives.

A. *Group under intensive treatment (one week each year)*

Table 1 shows the results obtained from 145 individuals, positive for microfilariae in January 1950 with an average rate of 79 microfilariae per 20 cmm. of blood, who were treated at that time with 2 mg. per kg. of body weight three times a day for one week. One year later, i.e. January, 1951, their blood was again examined and those still positive for microfilariae were again given the same treatment. In January, 1952, all 145 individuals were again examined and the positive persons re-treated. Table 1 indicates what can be accomplished by administering this dosage under careful supervision to a heavily infected population. The results one year following the second treatment show the greatest change and may be compared as follows with the results before treatment:

1) The percentage of positives was reduced from 100 to 17.5 per cent.
2) The average number of microfilariae per 20 cmm. of blood per person was reduced from 79 to 0.8.
3) Thirty-one, or 21.4 per cent, harbored from 1–5 microfilariae per 20 cmm.

of blood before treatment while 20, or 14 per cent, were in this group one year after the second treatment.

4) Fifteen were in the 6–10 group before treatment while only 3 were in this group one year after the second treatment.
5) Thirty-three were in the 11–30 group before treatment while only two were in this group one year after the second treatment.
6) Sixty-six had harbored more than 30 microfilariae per 20 cmm. of blood before treatment while no individuals harbored more than 30 microfilariae after two years.

For quantitative comparison of this group with experimental infection rates in mosquitoes, see Table 5.

B. *Results in the field (treatment one week each year)*

In the field it is not always possible to procure the same results as those obtained with the selected and carefully supervised group shown in Table 1, the main reasons being:

1) There are always certain persons moving from one area to another in the island, whom we may designate as "migrators." During a year's period, from 10 to 20 per cent of the population of a district may change residence. When the whole population is examined for microfilariae only once a year the migrators positive for microfilariae are not detected until the annual census. All migrators moving into a new district are now required to be examined immediately and the positives are treated.
2) A certain number of individuals refuse to take the diethylcarbamazine. The reasons given are variable, e.g., some dislike the reactions which at times develop; others object to taking Western medicine.
3) Certain individuals known to have serious illness were excused from taking treatment.

At the bottom of each table is listed the number of nontreated positive individuals in each year.

1. *Districts with mosquito control.* In Table 2 are summarized the results of field studies before treatment and one year after their last treatment in the combined Four Districts listed above and in Paea.

In Paea, diethylcarbamazine was first administered to a small group of the population in 1948 (Galliard *et al.*, 1949), the whole district having completed its first round of treatment by 1951. The figures for Paea in Table 2 represent the follow-up on individuals, of whom most of the positives had received two courses of diethylcarbamazine. When studies were first begun in Tahiti microfilarial counts were not made, the results being recorded merely as "positive" or "negative." It is, therefore, not possible in Paea to present results before treatment on the same basis as the results for 1952. It is known, however, in the first surveys in Upper Paea that 41 per cent of the population was positive for microfilariae. This is somewhat higher than the 37.2 per cent found in the four districts of Faaone, Hitiaa, Mahaena, and Tiarei.

When compared with the percentages "before treatment" in the Four Districts,

an increase is shown in the 1–5 level only, i.e., from 6.4 per cent before to 8.9 in the Four Districts and to 9.1 per cent in Paea. This was undoubtedly occasioned by reductions in the counts of certain carriers from the higher levels to the lower 1–5 level even though they did not become completely negative.

On the whole the results in the Four Districts are slightly superior to the results in Paea. This may be attributed to the fact that the microfilarial incidence was higher in Paea before treatment and that supervision has not been as strict in Paea as in the other Four Districts. It will also be noted that the number of

TABLE 2

Field studies in treating individuals positive for microfilariae in combined 4 districts (Faaone, Hitiaa, Mahaena, Tiarei) and in Paea with diethylcarbamazine given 3 times a day for one week each year

FREQUENCY DISTRIBUTION OF MICROFILARIAE PER 20 CMM.	FOUR DISTRICTS OF FAAONE, HITIAA, MAHAENA AND TIAREI				PAEA, ONE YEAR AFTER LAST TREATMENT, 1952	
	Before treatment, 1950		One year after second treatment, 1952			
	No.	%	No.	%	No.	%
0	524	62.8	740	81.0	874	79.4
1–5	53	6.4	82	8.9	101	9.1
6–10	29	3.5	32	3.5	37	3.4
11–30	58	6.9	29	3.3	36	3.3
31–50	55	6.6	10	1.1	17	1.5
51–100	34	4.0	10	1.1	15	1.4
100	82	9.8	10	1.1	21	1.9
Total	835	100.0	913	100.0	1100	100.0
Total +	311	37.2	173	19.0	226	21.0
Average number of microfilariae per 20 cmm.	36		4.6		4.6	
Positive migrators			26		53	
Positive refusals			10		32	
Positive excused			8		7	

migrators into Paea and the number of positives who refused treatment were greater than in the Four Districts.

2. *Maiao, without mosquito control.* Maiao constitutes a somewhat different picture from the Four Districts and Paea in Tahiti. It is a small island about sixty miles south-west of Tahiti with a population which varied from 141 to 174 in this study. Because of a pass which accomodates only small row boats or pirogues and makes transportation difficult, it was anticipated that the population would be relatively stable. Accordingly, it was selected as a filariasis experimental area in which diethylcarbamazine only would be administered without any mosquito control.

It will be observed that the survey before treatment shows the carrier rate to

be lower than in the districts from Tahiti reported in this study. Twenty-seven per cent of the population was positive for microfilariae with an average of 33 microfilariae per 20 cmm. for the population as a whole. The percentage with microfilariae per 20 cmm. at the various levels recorded, ranged from 1.8 per cent at the 6–10 level to 9 per cent at the 100+ level.

The first treatment administered was 2 mg. per kg. of body weight, three times a day for seven days, to positives only. One year later, all remaining positives were again treated. The 1951 check-up did not exhibit results that were superior to the previous year, 1950. This was caused partially, however, by five positive migrators coming to the island. Following the 1951 check-up it was decided to

TABLE 3

Field studies in treating population of Maiao with diethylcarbamazine

FREQUENCY DISTRIBUTION OF MICROFILARIAE PER 20 CMM. OF BLOOD	BEFORE TREATMENT, 1949		AFTER TREATMENT					
			1950		1951		1952	
	No.	%	No.	%	No.	%	No.	%
0	120	73.0	132	85.7	145	84.0	133	95.0
1–5	9	5.4	8	5.2	14	8.1	7	4.5
6–10	3	1.8	4	2.6	6	3.2	1	0.5
11–30	5	3.0	4	2.6	6	3.2	0	0.0
31–50	3	1.8	4	2.6	1	0.5	0	0.0
51–100	10	6.0	2	1.3	1	0.5	0	0.0
100+	15	9.0	0	0.0	1	0.5	0	0.0
Total	165		154		174		141	
Total+	43	27.0	22	14.0	29	16.0	8	5.0
Average number of microfilariae per 20 cmm.	33		9		4		0.2	
Positive migrators			1		5		0	
Positive refusals			0		0		1	
Positive excused			0		0		0	

change treatment to the one day a month schedule for the whole population. This schedule is described in subject C, below, of this paper. The 1952 follow-up shows a marked decrease in positives over the 1951 figures, the percentage of positive individuals being 5 and the average number of microfilariae per 20 cmm. of blood being 0.2. Only one person was positive in the 6–10 level and no positives were found above this level. It will be noted that there were no positive migrators but there was one positive refusal.

C. *Experiments with other treatment schedules*

Although the dosage schedule first recommended for the use of diethylcarbamazine, i.e., 2 mg. per kg. of body weight, three times a day for one week,

has been used once a year in most of the work in Tahiti, certain other schedules have been explored. Used in field studies are 2 mg. per kg. of body weight three times a day, one day a week; two days every 6 months; 4 days every 6 months; 4 days every 3 months; and 1 day each month. The last combined the two factors of being easy to administer and of producing results that appeared to be as good as any of the others and, at the end of a year, superior to the old dosage schedule of one week each year.

Table 4 shows a series of 55 persons positive for microfilariae who were treated by this schedule. It will be observed that the findings at the different levels before treatment show similar percentages of positive individuals to those in Table 1, the one exception being at the 100+ level which is 7.3 per cent instead of 22.7 per cent. Also the average number of microfilariae per 20 cmm. of blood is 33 instead of 79 as shown in Table 1.

TABLE 4

Carriers treated with diethylcarbamazine one day a month

FREQUENCY DISTRIBUTION OF MICROFILARIAE PER 20 cmm.	BEFORE		AFTER	
	No.	%	No.	%
0	0	0	50	91
1–5	13	23.6	3	5.4
6–10	6	10.9	0	0
11–30	15	27.0	2	3.6
31–50	10	18.2	0	0
51–100	7	13.0	0	0
100	4	7.3	0	0
Total +	55	100.0	5	9
Average no. of microfilariae	33		0.7	

The results during the period of from 12–18 months may be compared with the results either one year after the first treatment or one year after the second treatment in Table 1. They are greatly superior to the former and also better than the latter.

It may, therefore, be concluded that as long as the one day a month schedule is continued, better results may be expected than with the one week a year schedule. The crucial test will come after a period of perhaps 2 years, when the treatment is discontinued for a year and the results checked at the end of the third year.

D. *Future recommended schedule*

In the meantime in an area with the degree of microfilarial infection occurring in Tahiti, it is planned to combine the old and new schedules and use the following procedure when administering diethylcarbamazine to a new group or area.

1) Administer 2 mg. per kg. of body weight three times a day for one week to those positive for microfilariae.
2) Thereafter, administer 2 mg. per kg. of body weight three times a day,

one day each month to the whole population for 2 years or until the population is essentially negative for microfilariae.

II. Comparison of rates of microfilariae in man before and following diethylcarbamazine with experimental injection rates in mosquitoes fed on carriers in laboratory

As indicated under Materials and Methods of this paper, one of the objectives of the program was to obtain information concerning the infection rates of mosquitoes fed on carriers harboring different levels of microfilariae. It was hoped by procuring such quantitative data that a comparison might be made with microfilarial rates in man either before or after treatment and determinations

TABLE 5

Possible infection rates before and after diethylcarbamazine

MICROFILARIAE PER 20 CMM. OF BLOOD	INFECTION RATES OF MOSQUITOES FED ON CARRIERS (ROSEN)		POTENTIAL INFECTION FACTOR	POTENTIAL INFECTION RATES					
	Per cent of mosquitoes positive	Average no. developing larvae per pos. mosquito		145 positive		Four Districts		Maiao	
				Before	After	Before	After	Before	After
1–5	50.4	1.9	.96	20.5	13.4	6.1	8.5	5.2	4.3
6–10	No information								
11–30	82.3	4.1	3.4	77.2	5.1	23.5	11.2	10.2	0.0
31–50	89.3	5.3	4.7	74.7	0	31.0	5.1	8.5	0.0
51–100	93.1	9.3	8.7	60.0	0	34.8	9.6	52.2	0.0
100+	97.1	17.8	16.3	370.0	0	159.7	17.9	146.7	0.0
Totals				602.4	18.5	255.1	52.3	222.8	4.3

obtained concerning the reduction of microfilariae necessary in man to guarantee reduction of mosquito infection to that low point where transmission would cease to occur.

The first part of Table 5 shows the infection rates in mosquitoes fed on carriers harboring different levels of microfilariae (Rosen, 1952). If one multiplies the percentage of positive mosquitoes by the average number of infective larvae per positive mosquito and divides by 100, an arbitrary "infection factor" may be established. Then by multiplying this by the percentage of persons positive for microfilariae at each level in a given group an arbitrary "infection rate" per level or per group may be determined.

It should be emphasized that these two indices are highly theoretical and that the "infection factor" is procured from experimental results obtained by feeding mosquitoes in the laboratory. What happens in the field is not known and may vary because of a number of factors among which are:

1) Differences in percentages of carriers.
2) Differences of microfilarial rates in carriers.
3) Possible pathologic effect of parasitism with filarial larvae in infected mosquitoes.

4) Possible natural loss of larvae from heavily infected mosquitoes between feedings on man.

In Tahiti, prior to the institution of filariasis control measures, the percentage of mosquitoes caught in the field and found to be infected is certainly far below the percentages of positive mosquitoes found in the laboratory experiments listed. About ten per cent of the mosquitoes dissected from two control districts for ten months have been positive for developmental-stage larvae.

Using the "infection factor" described above to indicate a possible comparative factor of laboratory infections procurable at the different levels but also realizing that it does not necessarily apply to percentages found in the field, one may compute the "infection rates" possible before and after the use of diethylcarbamazine in different groups. Computations are made for the 145 positive individuals shown in Table 1, for the Field Results of the Four Districts in Table 2 and the results in Maiao summarized in Table 3. These are listed in Table 5. It is obvious that marked differences between the "before" and "after" results exist in the groups compared. Just how low an "infection rate" must be obtained in order to assure reduction of transmission to a nondangerous level can be determined only by future observation.

III. Evaluation of program

In evaluating a filariasis control program, one is confronted with the complex problem of determining which data present the most reliable comparisons. Three standards used most extensively in the work in Tahiti are reviewed as follows:

1) *Clinical rates.* Clinical data based on the presence of elephantiasis, of acute filarial fever or of attacks of lymphangitis are collected in preliminary surveys. A study based on the frequency and severity of attacks of lymphangitis is in progress both in experimental and control districts and follow-up studies on these patients for several years should be helpful in determining a significant clinical index. Since many years of recurrent and superimposed infection are required before elephantiasis is apparent, it is not practical to employ such clinical data in immediate evaluation of the experimental procedures used in the development of a control program. A comparison of the incidence of clinical manifestations at the beginning of a program with similar data collected five or ten years later should provide significant information.

2) *Microfilarial rates.* Because of the ease in procuring samples of peripheral blood for examination, the microfilarial rates are commonly used in investigating filariasis. The usual procedure is to examine thick smears of 20 cmm. samples each. One or more 20 cmm. samples may be examined, the percentage of positives increasing in direct proportion to the number of such samples examined. In this study one 20 cmm. sample has been routine. However, filarial infections, often with marked clinical signs, are known to occur without the presence of microfilariae in the blood stream, which illustrates the main disadvantage of this method. Even so, it is the only method available in evaluating drugs to be used as microfilaricides.

3) *Filarial larval index in mosquitoes.* Either the infective larval rate or the

developmental larval rate in mosquitoes should be of value in determining the progress of a program, provided one can dissect representative collections of mosquitoes from a community. When in possession of other representative data, either of the two rates mentioned above may be used to compute a transmission index such as the one suggested by Jachowski and Otto (1952). However, collecting such information is expensive and time consuming and may require the dissection of several thousand mosquitoes from an area to be tested. The minimum number of mosquitoes necessary to dissect and likewise the period of time over which it is necessary to make collections in order to compute a reliable index are still to be determined.

All three of the above mentioned procedures are used in Tahiti for the collection of information, but as yet no decision has been reached concerning their comparative value in determining the success of a filariasis control program. If and when any one of them can be considered adequate to signify the prevalence of filariasis in a given area, or to evaluate the progress of a control program, then that index may be accepted as a standard.

IV. Significance of reduction obtained

The results summarized in Tables 1 to 5, regardless of methods of computation, i.e., by (1) the percentage of people positive, (2) the average number of microfilariae per 20 cmm. of blood in a population group, (3) the frequency distribution of microfilariae at different levels, or (4) the potential infection rate in mosquitoes, show that the rates before treatment or in control areas in which no diethylcarbamazine has been administered, when compared with after treatment rates, all indicate marked reductions in those areas in which the drug has been used. These results, therefore, do not suggest that the use of diethylcarbamazine by the methods here described are in any instance associated with increased transmission.

They further show that the reductions are greatest in those groups in which the most thorough and careful administration of diethylcarbamazine was possible. Whether any of the reductions in microfilarial rates obtained in this study are sufficient to indicate that transmission has been lowered to that minimum level where new cases of clinical filariasis are no longer occurring can only be determined by future observation over a period of years.

SUMMARY

Annual microfilarial counts have been determined for periods of 1, 2 and 3 years following the use of diethylcarbamazine (Hetrazan, Notezine) as a microfilaricide in a filariasis control program. Two dosage schedules have been employed: (1) Two mg. per kg. of body weight 3 times a day for 7 days, repeated at the end of the year on the individuals who remained positive, (2) 2 mg. per kg. of body weight 3 times a day, 1 day each month for a period ranging from 12 to 18 months. The results are recorded in terms of (a) percentage of the population positive for microfilariae, (b) average number of microfilariae per 20 cmm. of blood, and (c) the frequency distribution in the population of microfilarial counts

per 20 cmm. of blood at six different levels. The results may be summarized as follows:

1. Of 145 positive individuals treated with schedule (1) the percentage of positives was reduced to 17.5 at the end of the second year; the average microfilarial count per 20 cmm. dropped from 79 to 0.8; and the frequency distribution results showed marked reductions at all levels, no positives occurring above the 11–30 group.

2. Field studies in areas where considerable migration of population occurs and where supervision of drug administration was not as rigid as in the above group, likewise exhibited marked reductions at the end of 2 years, but to a lesser extent.

3. The second dosage schedule of 2 mg. per kg. of body weight 3 times a day, 1 day a month, for 1 year gives promise of being a practical method of administrating diethylcarbamazine in a filariasis control program.

4. A possible "infection factor" based on the experimental results of Rosen in which mosquitoes were fed on man harboring different levels of microfilariae is suggested and a potential "infection rate" is determined from certain experiments in this program showing the frequency distribution at these same levels in man before and after the use of diethylcarbamazine.

5. All data observed following the administration of diethylcarbamazine as employed in this study show marked reduction in the microfilarial rates. Whether these reductions are sufficient to limit the transmission of *W. bancrofti* to a level where clinical cases will cease to occur can be determined only by observation over a period of years.

RESUME

Des examens de sang pour recherche de microfilaires ont été pratiqués chaque année, et 1, 2 et 3 ans après l'administration de diethylcarbamazine (Hetrazan, Notezine) utilisé en tant que microfilaricide au cours du programme de controle antifilarien. Deux méthodes de dosage ont été employées pour le traitement des porteurs de microfilaires. (1) Deux mg. par kilog. de poids 3 fois par jour pendant 7 jours, renouvelé à la fin de l'année pour les personnes restées positives, (2) 2 mg. par kilog. de poids 3 fois par jour, un jour chaque mois pendant 12 à 18 mois. Les résultats sont mesurés en fonction de a) Le pourcentage de porteurs de microfilaires, b) Le nombre moyen de microfilaires par 20 mm^3 de sang, c) La distribution des divers nombres de microfilaires correspondant à 6 catégories différentes. Les résultats ont été les suivantes:

1. Chez les 145 individus positifs traités par la méthode (1), à la fin de la deuxième année le pourcentage des positifs a été réduit à 17, 5 et le nombre moyen de microfilaires est tombé de 79 à 0, 8. La distribution des divers taux de microfilaires a montré des réductions marquées à tous les niveaux, aucun positif n'apparaissant au dessus de l'échelon 11–30 microfilaires.

2. Les études sur le terrain pratiquées dans les régions où se produit une migration considérable, et où la surveillance de l'administration du produit n'a pas été aussi stricte que pour le groupe sus mentionné, ont montré de même des nettes réductions à la fin de la 2ème année, quoique moins considérables.

3. La deuxième méthode, soit 2 mg. par kg. de poids 3 fois par jour, 1 jour chaque mois, pendant 1 an, semble devoir être une méthode pratique d'administration de l'hétrazan dans un programme de controle de la filariose.

4. "Des indices d'infection" possibles sont suggérés, basés sur les résultats expérimentaux de Rosen, suivant les infestations des moustiques nourris sur des donneurs à différents taux de microfilaires, et des "indices d'infection" potentiels sont calculés de même à partir de la fréquence de ces différents taux chez l'homme avant et après hetrazan.

5. Tous les résultats observés après l'administration de diethylcarbamazine au cours de cette étude, ont montré une réduction nette des taux de microfilaires. Seule une observation étendue sur plusieurs années permettra de déterminer si ces réductions sont suffisantes pour limiter la transmission de *W. bancrofti* à un niveau tel que n'apparaissent plus de nouveaux cas cliniques.

REFERENCES

Beye, H. K., Edgar, S. A., Mille, R., Kessel, J. F. and Bambridge, Ben, 1952. Preliminary observations on the Prevalence, clinical manifestations and control of filariasis in the Society Islands, *Am. J. Trop. Med. and Hyg.* **1**: 637–661.

Beye, H. K., Kessel, J. F., Heuls, J., Thooris, G., and Bambridge, Ben, 1953. Nouvelles recherches sur l'importance, les manifestations cliniques, et la lutte contre la filariose à Tahiti, Océanie Francaise, *Bulletin de la Société de Pathologie Exotique* **46**: 144–163.

Beye, H. K., South Pacific Commission Conference of Experts of Filariasis and Elephantiasis—Introductory paper: Control of Bancroftian filariasis in the South Pacific. In press.

Galliard, H. and Mille, R. 1949. Essais de traitement de la Filariose a *Wuchereria bancrofti* var. pacifica par 1-diethylcarbamyl, 4-methyl-piperazine a Tahiti, *Bul. Soc. Path. Exotique* **42**: 304–313.

Galliard, H., Mille, R. and Robinson, W. A. 1949. La Filariose à *Wuchereria bancrofti* var. pacifica a Tahiti et dans l'Archipel de la Societe, *Ann. de Parasit.* **24**: 30–48.

Hewitt, R. I., Hushner, S., Stewart, H. W., Wallace, W. S. and Subbarow, Y. 1947. Experimental chemotherapy of filariasis. III. Effect of 1-diethylcarbamyl, 4-methylpiperazine hydrochloride against naturally acquired filarial infections in cotton rats and dogs, *J. Lab. and Clin. Med.* **32**: 1293–1303.

Hewitt, R. I., Kenney, M., Chan, A. and Mohanud, H. 1950. Follow-up observations on the treatment of Bancroftian filariasis with Hetrazan in British Guiana, *Am. J. Trop. Med.* **30**: 217–237.

Hewitt, R. I., White, E., Hewitt, D. B., Hardy, S. M., Wallace, W. S. and Anduze, R. 1950a. The first year's results of a mass treatment program with Hetrazan for the control of Bancroftian filariasis on St. Croix, American Virgin Island, *Am. J. Trop. Med.* **30**: 443–452.

Jachowski, L. A. and Otto, G. F. 1952. Filariasis in American Samoa. II. Evidence of transmission outside of villages, *Am. J. Trop. Med. and Hyg.* **1**: 662–670.

Kenney, M. and Hewitt, R. I. 1949. Treatment of Bancroftian filariasis with Hetrazan in British Guiana, *Am. J. Trop. Med.* **29**: 89–114.

Oliver-Gonzalez, J., Santiago-Stevenson, D. and Maldonado, J. F. 1949. Treatment of filariasis bancrofti with Hetrazan, *J.A.M.A.* **139**: 308–309.

Rosen, L. 1952. Infection of *Aedes pseudoscutellaris* with *Wuchereria bancrofti* fed on man harboring different levels of microfilariae. Personal communication.

Santiago-Stevenson, D., Oliver-Gonzalez, J. and Hewitt, R. I. 1947. Treatment of filariasis bancrofti with 1-diethylcarbamyl, 4-methylpiperazine hydrochloride ("Hetrazan"), *J.A.M.A.* **135**: 708–712.

ON THE GROWTH OF *DIROFILARIA IMMITIS* IN THE MOSQUITO

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Considerable work has been done on the morphological changes attending filarial development in the mosquito but the literature contains scant information on filarial growth *per se*. Saisawa (1913) showed that *Dirofilaria immitis* attained a length of 161 μ by the 5th day in *Aedes aegypti* and *Anopheles maculipennis*. Brug (1931) presented data on development of *Wuchereria malayi* in two species of *Mansonia*. The parasite changed from a length of 100 μ on day 1 to 1300 μ on day 10. On the other hand, Feng (1936) indicated that *W. malayi* in *Anopheles hyrcanus sinensis* increased from 158 μ to 1304 μ between days 1 through 6. With *Wuchereria bancrofti*, it has been shown by Kobayashi (1940) that the parasite changes from a length of 206 μ on the 1st day to a length of 1617 μ by the 11th day in *Culex fatigans*. Kotcher (1941) reported that the frog filariid, *Folyella brachyoptera*, changed from 147 μ in length on the 1st day to 880 μ on the 18th day of growth in *Culex pipiens* and *C. quinquefasciatus*. Highby (1943) demonstrated that *Dipetalonema arbuta*, in various mosquitoes, grew from a length of 90 μ on the 3rd day to a maximum of 1.16 mm. on about the 9th day of incubation. This author also indicated that the larval stages varied considerably both in length and width.

That the host constitution has a profound effect upon the development of the filaria in the mosquito is the central hypothesis underlying an investigation discussed in an earlier publication (Kartman, 1953). The present report presents data on growth of filariae in the mosquito as an addition to that already recorded in the above paper. It should be noted that the data presented below are not offered as definitive evidence since they represent incidental observations gathered during experimental work which emphasized other features of the host-parasite relationship.

MATERIALS AND METHODS

A supply of microfilariae of *Dirofilaria immitis* was assured by use of an infected dog originally obtained from St. Augustine, Florida in 1945. The *Anopheles quadrimaculatus* were obtained from the Health and Safety Department, Tennessee Valley Authority, Wilson Dam, Alabama, and the *A. freeborni* came from the colony maintained at the National Institutes of Health malaria laboratory in Columbia, South Carolina. The *Culex quinquefasciatus* were from Galveston, Texas, and the *C. pipiens* from material collected near Baltimore. The *Aedes aegypti* came originally from the Laboratory of Tropical Diseases, National Institutes of Health, Bethesda, Md., and the *A. albopictus* were obtained

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from Philippine material maintained at the Army Medical Center in Washington, D. C.

Rearing and maintenance of adult mosquitoes were accomplished in an air-conditioned insectary held at approximately 27°C. and 85 to 90 per cent relative humidity. Female mosquitoes were fed on the infected dog while confined in lantern globe cages, or they were allowed to feed on it overnight in a large cage. Dissections of mosquitoes were made daily over a period of twenty days and measurements of the filaria larvae in them were recorded. These measurements were done with an ocular micrometer in a calibrated microscope and most material was measured while alive. Occasionally, vigorously moving third stage larvae or microfilariae were subjected to a drop of 2 per cent formalin run under the cover slip into the saline; measurements were made as soon as excessive movements subsided.

A more complete account of materials and methods used in these studies are given in the paper on the major portion of this investigation (Kartman, l.c.).

DATA

The microfilariae in the dog's blood varied in length from 238 μ to 294 μ, and in width from 4.9 μ to 6.6 μ. The mean values for lengths of different batches of microfilariae might range from about 259 μ to 273 μ, while the mean width usually gave a value of about 6 μ. The mean length of 100 microfilariae was 265 μ.

Figures 1, 2 and 3 present the mean lengths of *D. immitis* larvae during a twenty-day period in various mosquitoes. The curves are based on measurements of the following mean numbers of filaria larvae per day for the various host species: *Culex pipiens*, 6; *C. quinquefasciatus*, 8; *Culex* hybrid, 8; *Anopheles quadrimaculatus*, 19; *A. freeborni*, 17; *Aedes aegypti*, 7; and *A. albopictus*, 21. The considerable variation shown in the number of larvae measured per day per host species was due to the fact that certain mosquito species responded to the presence of the parasite by consistently restricting the number of developing worms to few or, in the case of many individual females, by eliminating them altogether (Kartman, l.c.).

Twenty-five worms per larval stage were measured after removal from a favorable host, *Anopheles quadrimaculatus*. The following mean lengths and mean widths respectively were obtained: "sausage" stage, 111 μ by 15 μ; second stage, 347 μ by 27 μ; third stage, 850 μ by 25 μ. The "sausage" stage varied from 98 μ to 196 μ long by 10 μ to 23 μ wide; the second stage, from 112 μ to 680 μ by 16 μ to 39 μ; and the third stage, from 700 μ to 1100 μ by 23 μ to 26 μ.

Although daily measurements were made on width of the filaria larvae no separate data are here presented since data on growth in length serve to indicate the essential type of growth of *D. immitis* in the mosquito host. The data on width have been used to obtain a picture of larval growth based on ratios of mean length to mean width and these data are shown in Table 1. It is seen that the greatest difference between length and width was found in the microfilarial stage in which the length was over 50 times greater than the width. In the "sausage" stage this difference was contracted to its smallest value since the length

varied from 5 to 11 times greater than the width. Thus the overall increase in length was very great, whereas the change in width became quite restricted espe-

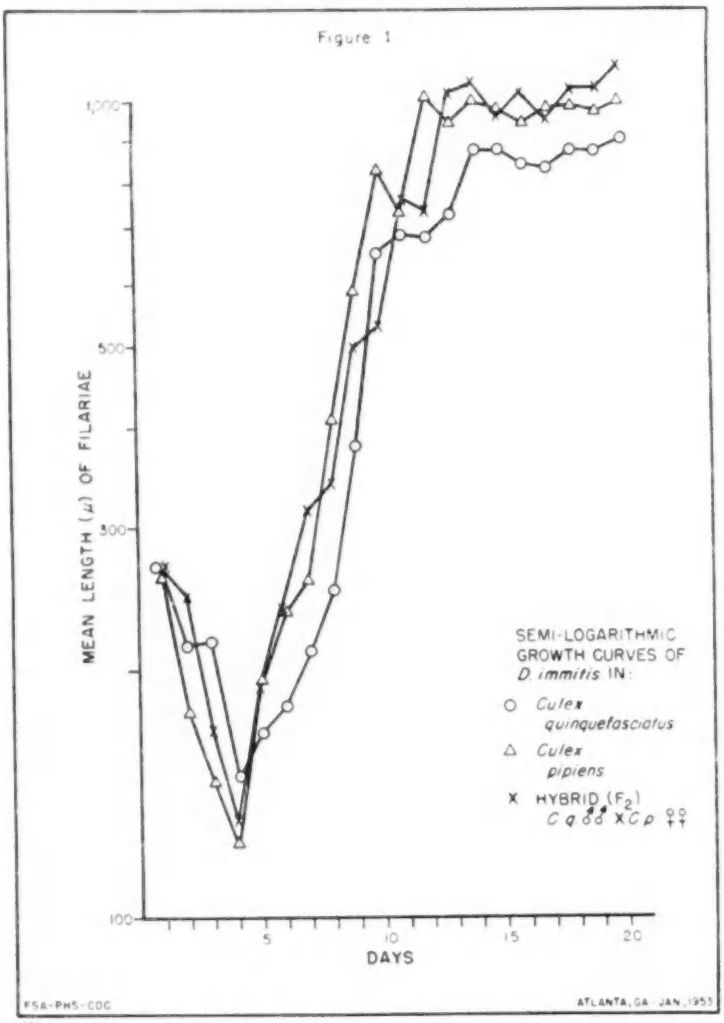

FIG. 1

cially after the third larval stage had been reached. In such hosts as *Culex pipiens*, *Aedes albopictus*, and *Anopheles quadrimaculatus* the length of the third stage larva averaged over 40 times greater than the average width toward the

termination of the developmental period under consideration. Notice should be taken of the differences in the ratios of the related *Culex* and *Aedes* species and the similarity of the anopheline ratios.

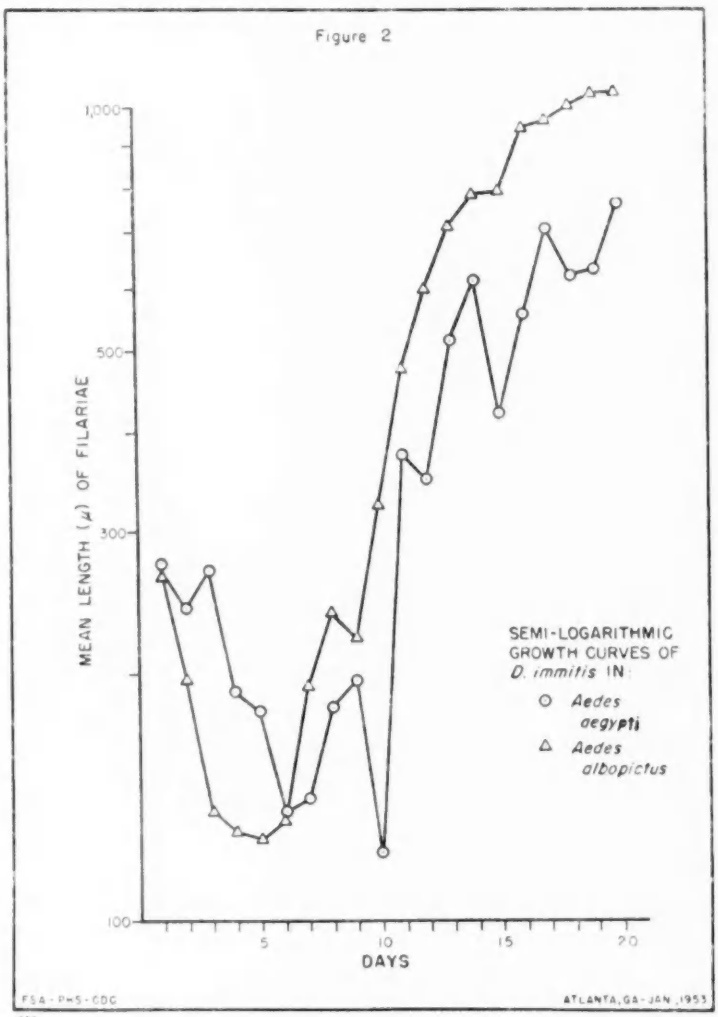

FIG. 2

Each larval stage underwent considerable growth and it is of interest to record growth of a single stage in more detail. Table 2 demonstrates changes in length in the third stage larva in three different mosquito species during the same time

interval. These measurements indicate a definite trend from smaller to larger mean lengths of third stage larvae in the *Aedes* and *Anopheles* species, whereas this tendency appears less evident in *Culex quinquefasciatus*. Frequency distribu-

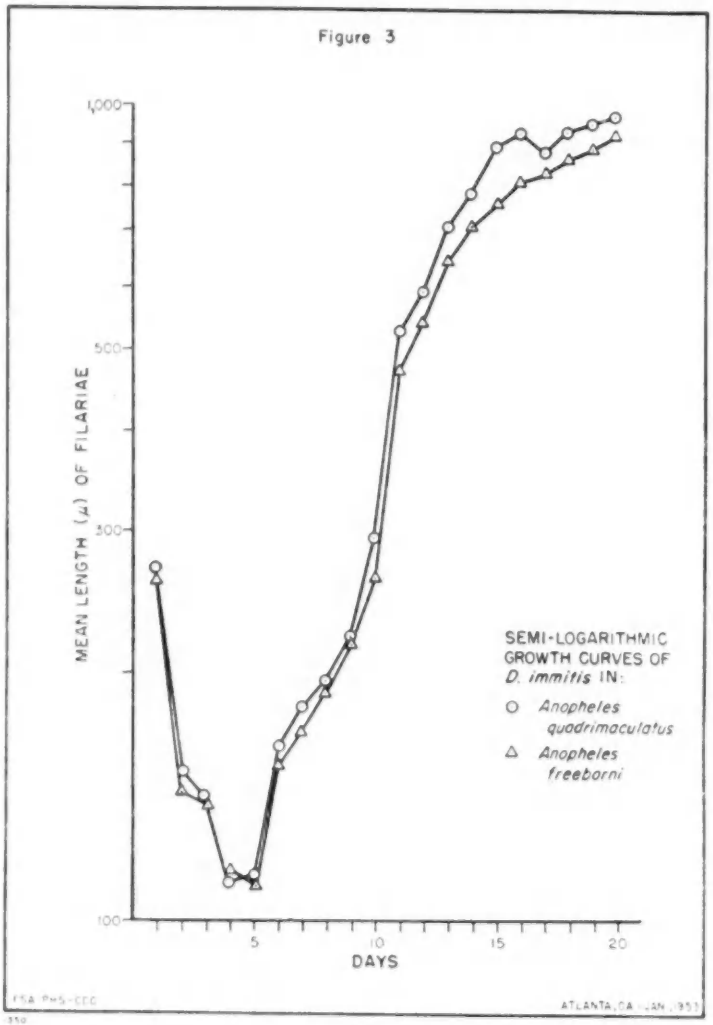

FIG. 3

tions of larval lengths indicate that from day 12 through day 14 about 88 per cent of third stage *D. immitis* larvae in *A. quadrimaculatus* were under 900 μ long. From the 15th to the 20th day 65 per cent of the larvae were 900 μ long or more

TABLE 1

Relation of growth in length to growth in width of D. immitis *in different mosquitoes**

DAYS IN MOSQUITO	RATIO OF MEAN LENGTH TO MEAN WIDTH (L/W) IN					
	Culex		*Aedes*		*Anopheles*	
	quinquefasciatus	*pipiens*	*aegypti*	*albopictus*	*quadrimaculatus*	*freeborni*
1	52	53	53	53	53	52
2	30	16	48	19	13	14
3	31	11	54	8	9	8
4	9	6	8	6	6	6
5	5	7	20	5	5	6
6	6	8	8	4	7	8
7	5	8	7	7	8	7
8	8	11	7	7	7	7
9	10	17	6	7	7	7
10	19	26	9	9	9	9
11	20	24	13	14	17	15
12	20	43	16	18	18	18
13	24	41	17	25	21	20
14	31	44	19	28	28	23
15	36	40	13	28	32	28
16	33	39	20	39	36	29
17	34	40	27	40	37	33
18	36	41	23	43	38	34
19	37	40	22	43	37	35
20	37	41	28	44	40	36

* Widths were obtained from the same worms measured for length and only the greatest widths were recorded; all values in microns; decimals have been discarded.

TABLE 2

Length of third stage larvae of D. immitis *as related to time in different mosquitoes*

DAYS IN MOSQUITO	MEAN LENGTH OF LARVAE IN INDICATED HOST* (MICRONS)		
	Culex quinquefasciatus	*Aedes albopictus*	*Anopheles quadrimaculatus*
12	—	750	731
13	867	793	826
14	880	886	873
15	888	957	976
16	885	955	1035
17	878	987	1020
18	875	1072	1065
19	889	1090	1075
20	898	1085	1092

* The number of larvae measured per day per host species follows: *C. quinquefasciatus*, 10; *A. albopictus*, 25; *A. quadrimaculatus*, 25. Decimals have been discarded.

and, of these, about one third were 1000 μ or greater. No measurements were recorded beyond the 20-day period to determine whether the growth of infective

larvae terminated at a maximum of about 1100 μ and whether those larvae measuring from 700 μ to 900 μ at 20 days subsequently increased their lengths.

DISCUSSION

The growth curves for *Dirofilaria immitis*, presented here, appear to have a similar form in each instance. Erratic filarial growth, as in *Aedes aegypti*, causes the curve to deviate from the "typical" shape. As indicated above, other species of filariae show a similar type of growth during their intra-culicid development. An exception to this apparently occurs in the growth of *Conspiculum quindiensis*, a filarial parasite of the lizard, *Calotes versicolor*. Pandit *et al.* (1929), presenting data on the growth of this worm in *Culex fatigans*, show an uninterrupted increase in length from microfilariae to infective larvae. No changes either in length or width suggesting the typical "sausage" stage are given, although mention is made of "quiescent tailed forms" in the thorax of the mosquito. This contradicts most observations on the growth of filaria larvae in mosquitoes and should be investigated more critically.

The present data suggest that significant variations in filarial growth occur in different mosquitoes under identical laboratory conditions. *Culex pipiens* allowed greater growth in length of the dog heartworm than did *C. quinquefasciatus*; and hybrids (F_2) of these two *Culex* species exhibited more growth vigor of the parasite than did *pipiens*. It is of interest to note that although *pipiens* was an unfavorable host for survivorship of the worms, if they did survive then it was a favorable host for their growth. This also appeared true for the *Culex* hybrids, but not to the same extent for *quinquefasciatus*. Although this would seem to suggest a conflict in the biological evidence the reasons for it have been partially elucidated (Kartman, l.c.). The differences between the *Aedes* species were quite striking, whereas the anopheline species showed very similar trends of filarial growth. Thus it is suggested that the taxonomic proximity of two mosquito hosts does not necessarily indicate that filarial growth within them will be similar.

On the basis of work with the cotton rat filariid, *Litomosoides carinii*, Williams (1948) has suggested that the infective larvae in the acarine host occurred in two definite lengths; ". . . 800 μ, which may develop into males, and 1000 μ, which perhaps develop into females." Bertram (1947) similarly found differences in the lengths of infective *L. carinii* larvae. As noted above, the infective larvae of *D. immitis* exhibited considerable variation in length and also showed profound growth in a favorable host. By the 20th day of incubation some third stage larvae were still short while the majority were over 900 μ long. This may be interpreted as a substantiation of the hypothesis that size is linked to sex of the larva. Nevertheless, the evidence of significant changes in length of third stage larvae would appear to demand further critical investigation aimed at elucidating the ultimate extent of such growth. It is of interest to note here that a trimodal frequency distribution was shown in the lengths of third stage larvae of *Dracunculus medinensis*. It was concluded that this was probably due to environmental influences rather than to sexual differentiation of the larvae (Moorthy, 1938).

SUMMARY

Generally similar growth curves of *Dirofilaria immitis* larvae were noted in the following experimental mosquito hosts: *Culex pipiens*, *C. quinquefasciatus*, and their F_2 hybrids; *Aedes aegypti* and *A. albopictus*; *Anopheles quadrimaculatus* and *A. freeborni*. The host constitution apparently had an effect upon the size attained by the filaria larvae, and the larval length/width ratio varied considerably between genera and also, in *Culex* and *Aedes*, between species, but to a lesser extent in *Anopheles*. Thus phylogenetic affinities between mosquito species do not necessarily imply similar growth of the dog filaria within them.

Third stage larvae undergo considerable change in length especially in favorable mosquito hosts. Nevertheless, at 20 days after the mosquito's infective meal the third stage filaria larvae still are to be found both as long and short individuals. Whether this is linked with sex differentiation is not known.

REFERENCES

BERTRAM, D. S., 1947, The period required by *Litomosoides carinii* to reach the infective stage in *Liponyssus bacoti*, and the duration of the mite's infectivity, *Ann. Trop. Med. Parasitol.* **41:** 253–261.

BRUG, S. L., 1931, Filariasis in the Dutch East Indies, *Proc. Roy. Soc. Med.* **24:** 663–674.

FENG, L. C., 1936, The development of *Microfilaria malayi* in *A. hyrcanus* var. *sinensis* Wied, *Chinese Med. J.*, Suppl. **1:** 345–367.

HIGHBY, P. R., 1943, Mosquito vectors and larval development of *Dipetalonema arbuta* Highby (Nematoda) from the porcupine, *Erethizon dorsatum*, *J. Parasitol.* **29:** 243–252.

KARTMAN, L., 1953, Factors influencing infection of the mosquito with *Dirofilaria immitis* (Leidy, 1856), *Exper. Parasitol.* **2:** 27–78.

KOBAYASHI, H., 1940, On the development of *Microfilaria bancrofti* in the body of the mosquito, (*Culex fatigans*), *Acta Jap. Med. Trop.* **2:** 63–88.

KOTCHER, E., 1941, Studies on the development of frog filariae, *Amer. J. Hyg.* **34**(D): 36–65.

MOORTHY, V. N., 1938, Observations on the development of *Dracunculus medinensis* larvae in *Cyclops*, *Amer. J. Hyg.* **27:** 437–460.

PANDIT, C. G., S. R. PANDIT AND S. IYER, 1929, The development of the filaria *Conspiculum quindiensis* in *Culex fatigans*, with a note on the transmission of the infection, *Ind. J. Med. Res.* **17:** 421–429.

SAISAWA, T., 1913, Untersuchungen uber Hundefilarien, *Zbl. Bakt. Orig.* **67:** 68–75.

WILLIAMS, R. W., 1948, Studies on the life cycle of *Litomosoides carinii*, filariid parasite of the cotton rat, *Sigmodon hispidus litoralis*, *J. Parasitol.* **34:** 24–43.

STUDIES ON THE EPIDEMIOLOGY OF THE HUMAN INTESTINAL FLUKE, *FASCIOLOPSIS BUSKI* (LANKESTER) IN CENTRAL THAILAND[1]

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I. INTRODUCTION

Infection with *Fasciolopsis buski* in Thailand is believed to be rare. To the best of our knowledge the only record of this infection is by Daengsvang and Mangalasmaya (1941) who reported having diagnosed five cases from Bang Kun Sri village in Dhonburi Province.

In September, 1952, a Thai female, 15 years old, was brought to a hospital in Bangkok with the chief complaint of abdominal pain, edema and low grade fever of one month duration. This girl had been a resident of Pak Hai District in Ayuthia Province since birth and had never visited Dhonburi Province. A stool examination revealed the presence of numerous *Fasciolopsis buski* eggs. Further quantitative determinations by the Stoll egg counting technique indicated an average of 88,000 eggs per ml. of formed feces. Since childhood she admitted having the habit of consuming raw water caltrops picked up on her way to school or work. Following hospitalization the patient vomited 29 adult flukes and passed 6 in her stools. In spite of supportive measures, blood transfusions, intravenous fluids and high protein diet, the patient developed general anasarca and died. At autopsy, 466 parasites were found in the lumen of the small intestine. Other interesting findings were congestion of the lungs, spleen, kidneys and liver. Degeneration of the liver cells with fat vacuoles in the cytoplasm was seen.

Owing to the great interest that this case stimulated from the epidemiological standpoint, the three siblings were examined for the presence of *Fasciolopsis* infection. Like their sister, they had never visited Dhonburi Province but were infected with this fluke. As a result of these findings, field trips were carried out to Pak Hai District in Ayuthia, and Bang Kun Sri Village in Dhonburi, where extensive cultivation of water caltrop (*Trapa bicornis*) was found to be present. It was then decided to make an epidemiological survey of these areas with primary consideration to infection with *Fasciolopsis*. After this study was begun, it was found that water caltrops were also cultivated around Prapraton village in Nakorn Phatom Province. The studies that form the basis of this report were therefore extended to this area as well.

[1] The authors wish to express their appreciation to Dr. Pramern Chandavimol, Chief of the Division of Communicable Disease Control, Thai Department of Health, for the constant and sympathetic interest in the project. The authors are also indebted to Dr. Vikit Virunavatti, Department of Medicine, Siriraj University Hospital, for bringing to their attention one of his patients with fasciolopsiasis.

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Map I

MAP OF THAILAND

II. DESCRIPTION OF THE LOCALITY

Ayuthia, Dhonburi and Nakorn Phatom Provinces are situated in Central Thailand, within a radius of 60 miles from Bangkok (Map 1). Central Thailand is a vast alluvial plain of the Chao Phaya river. The level of the land is only very little higher than that of the sea. During the rainy season this area appears as one great lake, interrupted by a few roads, scattered temples and numerous dwellings.

During the dry season, tidal influence is plainly evident as far as 50 miles from

the mouth of the river, near Bangkok. The rich soil of Central Thailand, its abundance of water-courses, both natural and artificial, and its comparatively dense population combine to make it one of the most eminently suitable areas of the world for the production of rice. Although there are a few roads reaching some districts and large villages, the principal means of communication is by boat.

The climate is tropical and humid with slight range of extremes. The climatological data of one meteorological station near to these districts (Don Muang) are essentially typical of the three provinces. They are summarized in Table 1. The average temperature throughout the year preceding that in which this study was carried out, was between 25.9°C. in January to 31.4° in April, which is the optimum for the development of the free stages of *Fasciolopsis buski*. The minimum mean temperature was 20.1°C. in January, and the maximum 37.3° in

TABLE 1

Summary of climatological data in Don Muang during 1951*

MONTH	MEAN AIR TEMPERATURE IN C°			RAINFALL IN MM.	MEAN RELATIVE HUMIDITY PER CENT
	Maximum	Minimum	$\frac{\text{Max} + \text{Min}}{2}$		
January	31.6	20.1	25.9	0.2	70.8
February	33.7	21.3	27.5	0	70.2
March	35.4	23.6	29.5	2.2	70.4
April	37.3	25.4	31.4	43.5	68.1
May	35.0	24.8	29.9	144.3	77.3
June	33.0	25.1	29.0	159.4	80.1
July	32.9	24.7	28.8	231.3	80.6
August	32.9	25.4	29.1	211.8	77.5
September	32.6	25.3	28.9	337.5	80.6
October	31.4	24.6	28.0	581.4	80.5
November	30.9	24.2	27.6	84.6	80.1
December	31.3	21.1	26.2	0.6	78.4

* Nearest meteorological station to the areas discussed.

April. Considering that "a drop to 70°F. (21°C). holds back early stages, but allows eggs already in the miracidium stage to go on hatching" (Barlow, 1925), even this minimum seems to be compatible with the survival of this parasite. The annual rainfall amounted to about 1,800 mm. and the relative humidity varied from 70.2 per cent in February to 80.6 per cent in July and September.

III. MATERIALS AND METHODS

In each locality, a reconnaissance was first made on a light skiff, and a schematic map was drawn. The localities where water caltrop was being cultivated were plotted on the maps.

Through the "Headman" of each village, the Bhuddist priests and the school teachers, arrangements were made for the people in the areas surveyed to deliver fresh stool specimens the morning following the day in which they received tin cans labeled with their name, age and sex. This information was recorded at the

time of collection on a punch card designed by the senior author. Other information such as appearance and consistency of the stool, method of examination, parasitic findings, egg counts, etc., were filled out at the time of examination. Between 80 and 90 per cent of the people who were solicited delivered specimens. The findings reported were based on a single stool specimen examined by two direct smears. Whenever possible, stools positive for *Fasciolopsis* were examined again by the Stoll dilution egg-counting technique as given by Cort, Grant and Stoll (1926). All egg counts were reduced to the basis of formed feces, and in children up to 4 years they were further reduced to 50 per cent because of the small size.

IV. RESULTS

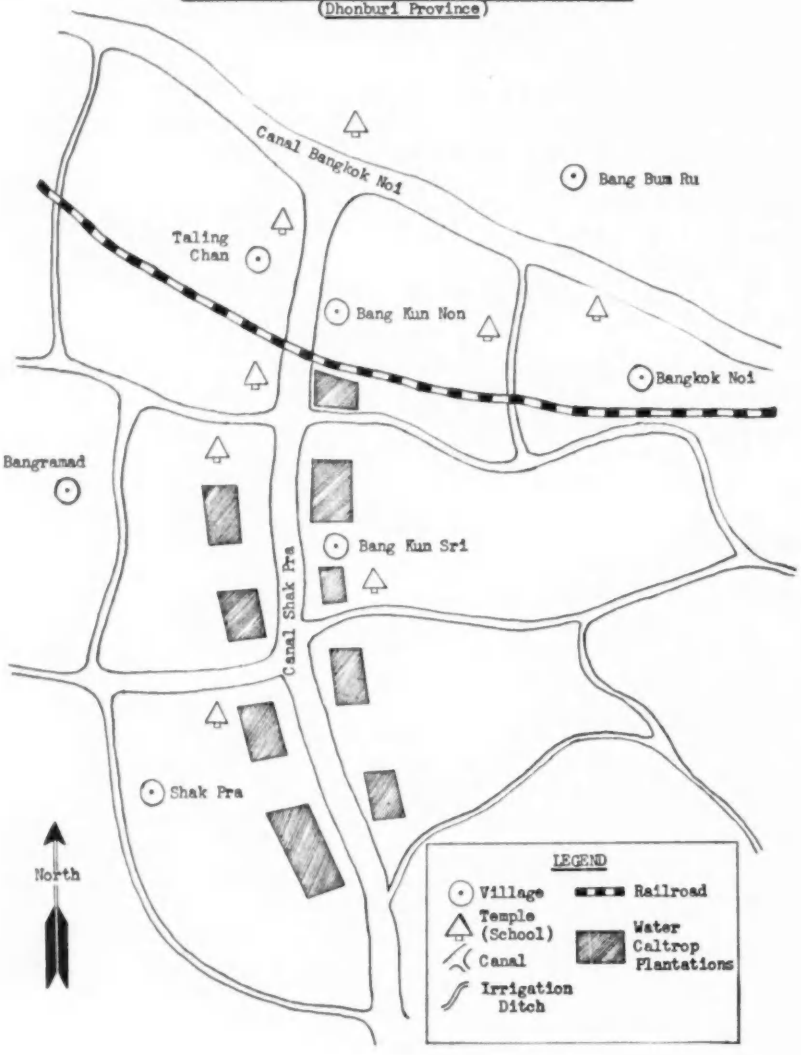
Bang Kun Sri Area

The area in and around Bang Kun Sri (Map 2) consists of flat country most of which is under water for the greater part of the year. It is situated at a distance of about ten miles west of Bangkok. The strips of land above water level are used primarily for the cultivation of fruits and sugar cane, those under water are used for the cultivation of water caltrops and lotus (Figure 1 and 2). Although no recent census is available, it is estimated that approximately 3,000 people live in this area. The majority of the population are engaged in agriculture. The remainder are engaged in commerce to and from Bangkok, or are domestics and laborers.

The dwellings are built on stilts anchored under the water level and people move around mostly by skiffs or canoes. Ducks are raised by some families and are frequently seen swimming in the canals or resting and splashing in muddy pools. Larger domestic animals such as pigs and water buffaloes were not seen in any of these villages. No service for water supply, sewage and refuse disposal is organized. Rainwater is the only source of safe drinking water. Garbage is dumped promiscuously in the canals. Pit latrines are uncommon. When present they are situated above the water level so that excreta are dropped directly into the water. Children usually defecate directly in the canal or irrigation ditches. Defecation seldom takes place directly in the water caltrop plantations since this habit would be considered "unclean" by the farmers who must bathe frequently in the ditches in order to attend to the cultivation of this fruit. For the same reason human or animal fertilizers are not used.

A coprological examination of 802 unselected individuals of both sexes was made in six villages south of canal Bangkok Noi. The results of this examination are summarized in Table 2. Between 8 and 25 per cent of the persons examined in the various villages were found to pass eggs of *Fasciolopsis* in their stools with an overall incidence of 18 per cent. Attention is called to the fact that in Bang Kun Sri and Shak Pra, where people live in the immediate vicinity of fields in which water caltrops are cultivated (Map 2), the incidence was 25 and 23 per cent, respectively. In other villages, a little farther removed from the site of plantations, the incidence varied from 8 to 10 per cent.

Map II
SCHEMATIC MAP OF BANG KUN SRI VILLAGE AND ENVIRONS
(Dhonburi Province)



Pak Hai District

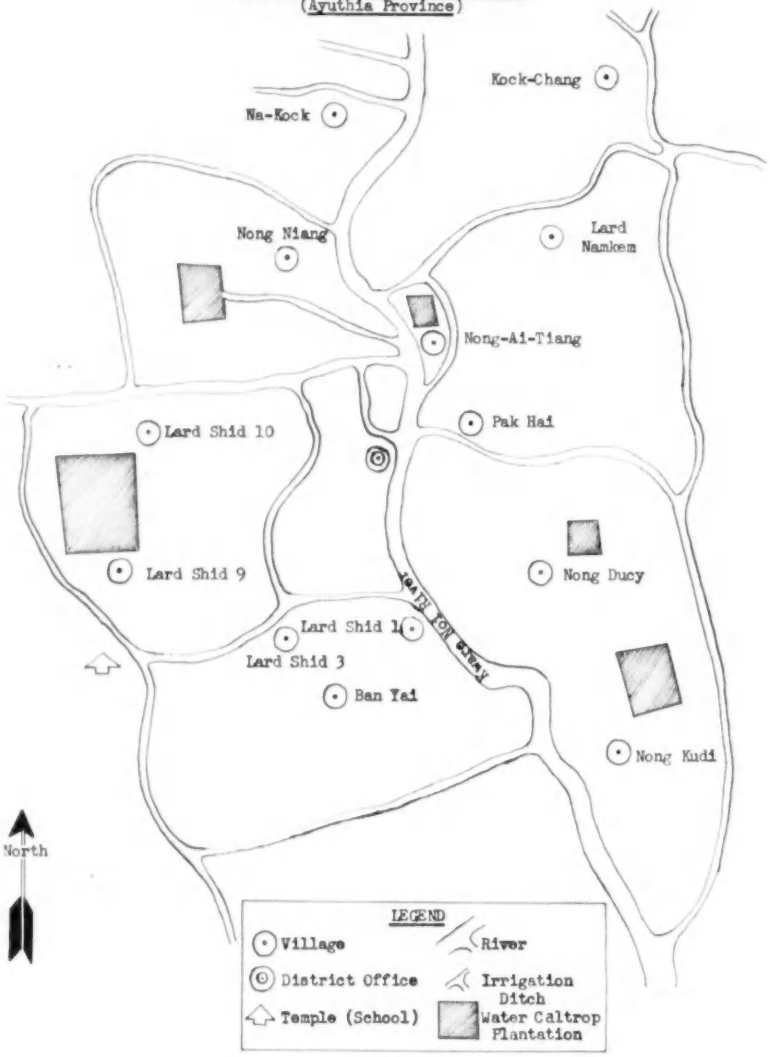
Pak Hai district (Map 3) is situated at a distance of about twenty miles northwest of Ayuthia, the ancient capital of Thailand. It is approximately fifty miles from Bangkok. Most of the villages lie along the Kware Noi River. The type of dwellings and the habits of the people are much like those described above. The

FIG. 1. Area near Bang Kun Sri used for the cultivation of water caltrops

FIG. 2. Close view of water caltrops in their natural habitat

dwellings of these communities are widely scattered along both sides of the canals and irrigation ditches, each community forming a small aggregation of isolated houses rather than true villages. The type of cultivation is not essentially

Map III
SCHEMATIC MAP OF PAK HAI DISTRICT
(Ayuthia Province)



different from that of the area described above except for the presence of numerous paddy fields. To work the fields, farmers keep water buffaloes which are usually sheltered under the same roof with the farmer's family. As described above, the houses are built on stilts anchored under the water level. It is estimated that approximately 10,000 people live in this district.

A coprological examination of 354 unselected individuals of both sexes was made in two villages. In one of them, Lard Shid 9, lived the family of the girl that died in Bangkok as a result of a severe infection with *Fasciolopsis buski*. Lard Shid 10 is situated on the opposite side of the same water caltrop plantation. As indicated in Table 2, 19 per cent of the people in Lard Shid 9, and 10 per cent of those in Lard Shid 10 were infected with *Fasciolopsis buski*.

Prapraton area

The area in and around Prapraton village (Map 4) is situated at a distance of about 35 miles southwest of Bangkok. The land is used primarily for the cultiva-

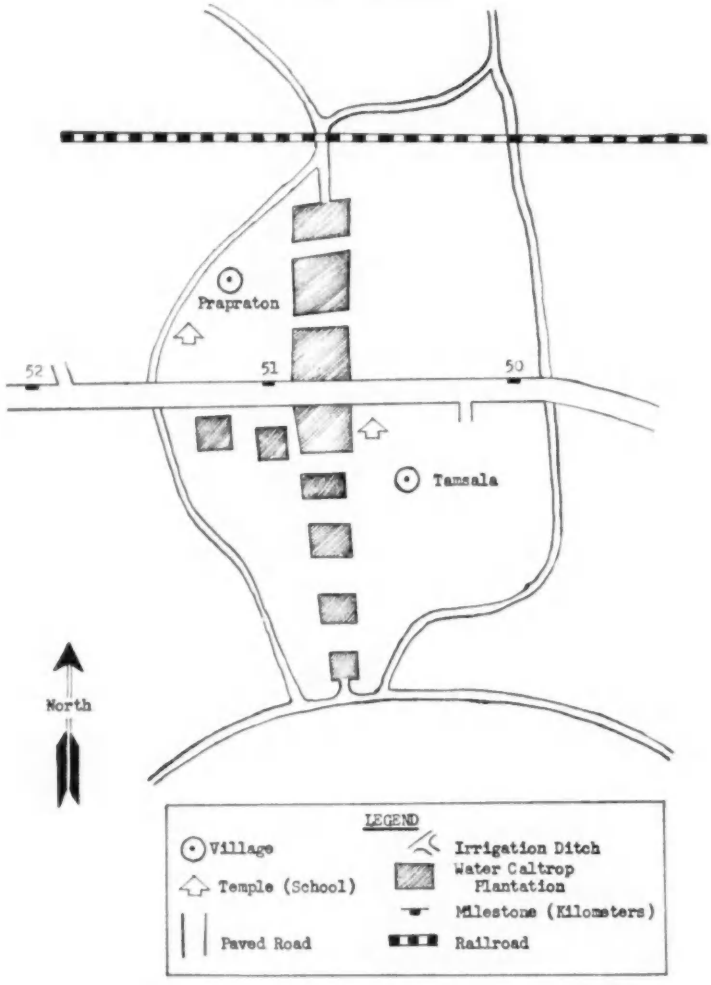
TABLE 2

The incidence of infection with Fasciolopsis buski *according to locality*

LOCALITY	NUMBER EXAMINED	NUMBER POSITIVE	PER CENT POSITIVE
Bang Kun Sri	269	67	25
Shak Pra	214	50	23
Bang Kun Non	41	4	10
Taling Chan	173	18	10
Bang Ramad	56	5	9
Bangkok Noi	49	4	8
Total	802	148	
Average per cent			18
Lard Shid 9	151	33	19
Lard Shid 10	203	21	10
Total	354	54	
Average per cent			15
Prapraton	169	2	1
Tamsala	238	4	2
Total	407	6	
Average per cent			1

tion of rice. In addition to rice, water caltrops, lotus and citrus fruits are raised. It is estimated that approximately 3,000 people live in this area. A railroad and a good highway pass through this district. Unlike the locations previously described, most of the land around Prapraton is almost always above the water level. About 90 per cent of the population are engaged in the rice cultivation. The remainder are domestics or laborers. The dwellings are built on stilts anchored in the ground. Water buffaloes, pigs, dogs, chickens and a few ducks are seen under the houses. As in the areas previously described, no service for water supply, sewage and refuse disposal is organized. Rain water is the only source of safe drinking water. Some pit latrines are available but seldom used. As a rule, defeca-

Map IV
SCHEMATIC MAP OF PRAPRATON VILLAGE AND ENVIRONS
(Nakorn Patom Province)



tion takes place anywhere in the fields except where rice and water caltrop are cultivated since farmers would regard this habit "unclean."

A coprological examination of 407 unselected individuals of both sexes was made in Prapraton and Tamsala Villages. As indicated in Table 2, only six persons were found to pass eggs of *Fasciolopsis*. No significant difference in the incidence of this infection was found to occur in the two villages examined.

V. ANALYSIS OF INCIDENCE IN RESPECT TO TWO TYPES OF POLLUTION AREAS

In the three areas reported, there were two general types of situations with relation to soil pollution, (1) those in which pollution occurred directly in the water, and (2) those in which defecation occurred under fruit trees or anywhere on the ground away from water. Of the three places studied, Bang Kun Sri area and Pak Hai district were practically covered by water and it would have been necessary to travel a distance from the dwellings in order to find land above the water level. The opposite was true for Prapraton area. It is possible that the

TABLE 3

The incidence of infection with hookworm in the localities surveyed for Fasciolopsis buski

LOCALITY	NUMBER EXAMINED	NUMBER POSITIVE	PER CENT POSITIVE
Bang Kun Sri	269	12	4
Shak Pra	214	5	2
Bang Kun Non	41	3	7
Taling Chan	173	10	6
Bang Ramad	56	5	9
Bangkok Noi	49	2	4
Total	802	37	
Average per cent			5
Lard Shid 9	151	2	1
Lard Shid 10	203	0	
Total	354	2	
Average per cent			1
Prapraton	169	37	22
Tamsala	238	46	19
Total	407	83	
Average per cent			20

considerable difference in the rate of fasciolopsiasis in the two types of areas was due to this fact (Table 2).

To gather additional evidence on the differences in the two pollution areas, a comparison was made of the incidence of hookworm in the villages studied. As indicated in Table 3, in the areas predominantly under water where the incidence of *Fasciolopsis* was high (Bang Kun Sri and Pak Hai), the incidence of hookworm varied from zero to 9 per cent. Out of 1,156 individuals examined in the two areas, hookworm eggs were found only in 39, equal to 3 per cent. On the other hand, out of 409 persons examined in Prapraton and Tamsala villages, where the incidence of *Fasciolopsis* was very low, 83, equal to 20 per cent, had hookworm eggs in their stools in sufficient amount to be detected by direct smears. In view of the fact that hookworm infection is not possible when defecation takes place

in the water, these results are presented as suggestive evidence of the greater amount of water pollution in Bang Kun Sri and Pak Hai districts, and of soil pollution in Prapraton area.

VI. ANALYSIS OF INCIDENCE WITH RESPECT TO DISTANCE FROM WATER CALTROP PLANTATIONS

Although metacercariae of *Fasciolopsis buski* do not withstand drying, the common habit of dipping water caltrops in water from time to time to retain their freshness (Figure 3) could be conducive to spread of infection to areas where these plants are not cultivated. In Bang Kun Sri area it was observed that the

FIG. 3. Woman dipping water caltrops in the canal to retain their freshness

incidence of fasciolopsiasis among people living in the immediate vicinity of water caltrop plantations was much higher than in villages a few hundred yards removed from the site of plantations.

To check further the effect of distance from water caltrop plantations on the incidence of infectivity, 1,373 students and government employees were examined in Bangkok and Ayuthia where water caltrops from Bang Kun Sri and Pak Hai areas are sold on the streets. Although these people examined lived between 10 and 20 miles from the infected areas reported above, they had never visited them. Many of them, however, admitted having bought and eaten the fruits sold by peddlers in the streets or at the market. Of the 1,373 people examined, 863 were living in Bangkok, and 510 in Ayuthia. *Fasciolopsis* eggs were not found in the feces of any of them. These results are presented as a further suggestion that the great bulk of infection takes place in the localities where water caltrops are

cultivated and that the consumption of this fruit at a distance from the plantations has little, if any, epidemiological significance. Numerous interviews revealed that the native population prefers eating water caltrops after boiling. Peddlers are often seen in the streets provided with a charcoal boiler in which they cook the fruits. At the market, the fruits are sold raw to housewives who usually boil them at home. It is only in areas where the caltrops are cultivated that people working or playing in the fields eat the fruit raw, as they are easily gathered. This is especially true of the children (Figure 4).

FIG. 4. Child eating raw water caltrops while the parents harvest the fruits.

Attention should be called to the fact that lotus plants are cultivated in the same general areas and that Barlow (1925) suspected that they might also play a minor role in the transmission of the disease. People were frequently seen eating fresh stems of the lotus after peeling them. Since peeling is done by hand, it is possible that some metacercariae are carried by contaminated hands to the portion eaten.

VII. ANALYSIS OF INFECTION WITH RESPECT TO AGE AND SEX

As indicated in Table 4, a total of 1,563 persons were examined in the three areas. In this figure are included 784 males and 779 females with an unusually good representation of the younger age groups. The examination by simple smears gave 86 males (11 per cent), and 122 females (16 per cent) positive for *Fasciolopsis*. The incidence varied from 8 per cent among adults 15 years and over, to 15 per cent in children 5 to 14 years old, with an average for all the age groups of 13 per cent.

The average egg count of the persons harboring this parasite was 5,100 eggs per ml., with the average for the males at 6,600 and for the females at 4,000 (Table 5). From counts made of weighed specimens of stool from a patient known to harbor but one fluke, Barlow (l.c.) estimated that a mature adult produced from 10,000 to 15,000 eggs per day. Stoll and Kwei (1927) reported that each mature worm lays about 25,000 eggs per day. The patient seen at a Bangkok Hospital passed on the average 88,000 eggs per ml. of formed feces, and was found to harbor 501 flukes. Although the total amount of feces passed during a 24 hour period could not be accurately measured in view of the fact that the

TABLE 4

The incidence of infection with Fasciolopsis buski *according to age and sex*

AGE GROUP	MALES			FEMALES			BOTH SEXES		
	No. examined	No. infected	% infected	No. examined	No. infected	% infected	No. examined	No. infected	% infected
1–4	62	5	8	53	6	11	115	11	10
5–9	226	32	14	228	38	17	454	70	15
10–14	372	40	11	344	64	19	716	104	15
15+	124	9	7	154	14	10	278	23	8
All ages	784	86	11	779	122	16	1563	208	13

TABLE 5

The degree of infection with Fasciolopsis buski *according to age and sex*

AGE GROUP	MALES		FEMALES		BOTH SEXES	
	No. egg counts	Average egg count per ml	No. egg counts	Average egg count per ml	No. egg counts	Average egg count per ml
1–4	2	800*	5	2,000*	7	1,700*
5–9	16	5,200	23	4,100	39	4,500
10–14	25	9,000	35	4,900	60	6,600
15+	8	3,500	11	1,900	19	2,600
All ages	51	6,600	74	4,000	125	5,100

* These figures show a 50% reduction of counts for stool size in children 1 to 4 years old

patient had uncontrollable bowel movements, our rough estimate seems to be somewhere between those of the above authors.

Both in males and females the severity of infection increased up to 10 to 14 years of age and decreased in older groups. This difference of incidence and severity of infection in the various age groups might be explained by the differences in activity. The preschool children (1 to 4 years) are largely confined to the area surrounding the house and seldom get into the water to collect water caltrops. The 5 to 14 year group represents the school age. As indicated in Maps 2, 3, and 4, water caltrop plantations are often found between dwellings and the schools. As it was admitted by the patient that died in Bangkok and by other children interviewed, fresh water caltrops are often picked up by the children on

their way to and from school. In the older and more mature group (15 years and over) this practice does not occur so frequently since it would be regarded as "stealing" by the other members of the community. Their main source of infection would probably derive from being directly engaged in the cultivation, harvesting and selling of water caltrops.

VIII. THE RESERVOIR HOSTS RELATED TO THE DISTRIBUTION OF FASCIOLOPSIS INFECTION

As indicated by Barlow (1925) pigs and water buffaloes have been implicated as hosts of *Fasciolopsis buski*. In his experience, however, "no pig cases have been found in over six year's search" in areas where a very high degree of infection was present among human beings. He also never found water buffaloes infected with this fluke. In Thailand, the incidence with *Fasciolopsis* among human beings was greatest in Bang Kun Sri area where neither pigs nor water buffaloes were ever seen. Conversely, of the areas studied, the incidence was lowest in Prapraton Village and environs, where both of these mammals were commonly encountered. Although no attempts were made to examine the feces of these animals for the presence of *Fasciolopsis*, the above considerations seem to indicate that these animals need be considered little, if at all, in this region in connection with the epidemiology of this infection. Chickens and ducks do not need to be taken into account considering what we know about the biology of this parasite. In other words, in Central Thailand man alone seems to be the important definitive host and the reservoir of infection.

The water caltrop is recognized as the vegetable most commonly involved in human infection with the intestinal fluke in other parts of the world. If we compare the different districts, the location of water caltrop beds, the incidence of infection and the histories of food consumption, an obvious coincidence is found to exist also in Thailand.

An attempt was made to find the encysted metacercariae attached to the capsules of caltrops but with negative results, due to partial drying of the material while being transported to Bangkok, a day's journey by car, launch and skiff. A second attempt was also fruitless and further investigations along this line will be made as soon as conditions are suitable.

With respect to the molluscan hosts, snails were found on the under-surface of leaves in water caltrop plantations, on the bottom of the ponds just below the mud surface, and on logs that were occasionally present in the water caltrop plantations. Some were collected and brought to Bangkok for identification. Dr. Kloom Vacharobol, Professor of Zoology at Chulalongkorn University, identified them as *Limnaea pignis* (*sulvaria*), *Lymnaea plicatula* and *Caridia teruicostata*. A few snails were observed under the microscope but no cercariae were seen. Snails were rather abundant although not always easy to collect, since they dropped to the muddy bottom of the canals when disturbed.

IX. PROPHYLACTIC ASPECTS OF FASCIOLOPSIASIS IN THAILAND

Fasciolopsiasis in Thailand is not a rare condition as was believed heretofore. Our observations have demonstrated that there are endemic centers where this

infection is found in as many as 25 per cent of the population. It is impossible at the present time to state whether the endemic centers are limited to Central Thailand or whether other pockets of infection exist elsewhere in the country. For the same reason it is impossible to estimate how much physical disability and economic loss are produced in Thailand by fasciolopsiasis. One interesting finding of the survey here reported is that the endemic areas discovered are all limited in size and easily circumscribed. They can all be reached within one day's travel from Bangkok. Since water caltrops are cultivated in many parts of Thailand it seems possible that as studies are extended to other areas, new endemic centers may be discovered. Although we did not find any infection originating outside of the places where these fruits are being raised, one cannot exclude the possibility that a few infections may take place at some distance from endemic areas.

In Thailand there are certain conditions which make the prophylaxis of fasciolopsiasis quite different from what it is in China. Of these, perhaps the most important difference is in the method of water pollution. Whereas in China the custom is to preserve human excreta in various containers to be spread upon the fields, in Thailand human feces are not used as fertilizer. Water pollution takes place only because latrines are built over the canals, or because of promiscuous defecation in the canals. Whereas to control this infection in China "the more fundamental problem consists in the sterilization of night-soil" (Faust, 1939), in Thailand attention must be focused on other stages in the life cycle of the parasite as a possible point of attack.

SUMMARY AND CONCLUSIONS

A total of 2,936 persons was examined in Central Thailand for the presence of *Fasciolopsis buski*. The results support the conclusion that this infection is endemic in the examined areas where water caltrops (*Trapa bicornis*) are cultivated. A correlation between the degree of water pollution and incidence of infection was observed in different districts. The highest incidence and intensity of infection was found among children 10 to 14 years of age. To the authors' knowledge this is the first time that fasciolopsiasis has been reported as endemic in Thailand.

REFERENCES

Barlow, C. H., 1925. The life cycle of the human intestinal fluke, *Fasciolopsis buski* (Lankester), *Am. J. Hyg. Monog. Series* **4**: 1–98.

Cort, W. W., Grant, J. B., Stoll, N. R., and other collaborators, 1926. Researches on hookworm in China, *Am. J. Hyg. Monog. Series* **7**: 1–398.

Daensvang, S., and Mangalasmaya, M., 1941. A record of some cases of human infestation with *Fasciolopsis buski* occurring in Thailand, *Ann. Trop. Med. and Parasitol.* **35**: 43–44.

Faust, E. C., 1939. *Human Helminthology*. Second Edition. Lea and Febiger, Philadelphia.

Stoll, N. R., and Kwei, W. S., 1927. Egg-worm correlations in cases of *Fasciolopsis buski*, *J. Parasitol.* **13**: 166–172.

CLINICAL AND PATHOLOGICAL OBSERVATIONS ON SIXTY-FIVE CASES OF VIPER BITE IN ISRAEL

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The present article consists of a clinical study of 61 cases of viper bite in Upper Galilee and the Jordan Valley as well as an anatomical and histological examination of four additional cases which were observed at different hospitals in the Tel-Aviv region.

According to the opinion of the zoologists, all the poisonous snakes of this country belong to the family Viperidae. Cases of snake bite by the family Colubridae are unknown. There is still a difference of opinion as to whether poisonous snakes of the latter family exist in Israel. The most common types of snakes and their distribution are as follows:

1. *Vipera palestinae* in the center of the country and in the north.
2. *Echis coloratus* in the Judean Desert.
3. *Cerastes cerastes* in the south.

Considering the geographical regions in which the cases occurred and the snakes which were identified (33 in number) it appears that our material applies only to bites by the Palestinian viper. In Table 1 the statistical data of all of our cases are assembled.

VIPER VENOM AND ITS ABSORPTION

According to the literature, snake venom contains proteins (albumin and globulin), proteoses and peptones, enzymes, salts, etc. Previously it was thought that the actual poison was linked to the protein and peptone fractions, but lately it has become possible to isolate two specific toxins—ophiotoxin ($C_{17}H_{26}O_{10}$) from cobra venom and crotalotoxin ($C_{34}H_{54}O_{21}$) from viper venom. These substances belong to the nitrogen free glucosides of the saponines.

According to our clinical observations we have been of the opinion that snake venom is absorbed and spreads via the lymph channels. This is proved, among other things, by its slow absorption, the toxic lymphangitis and the lymphadenitis which developed in 11 cases only a few hours after the bite, and the widespread hemorrhages which spread along the lymph channels. Cases coming to postmortem also showed, at times, this distribution of hemorrhages along the lymph channels and around the regional lymph nodes.

In the literature we also found experimental support for this assumption. According to G. Duran-Reynals (1939), snake venom, especially of the viperae, contains a factor which promotes its penetration into the tissues, in particular the connective tissue and the capillaries. This factor is not necessarily identical

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with the toxic element of the venom. Intravenous injection of snake venom into rabbits intensifies the spread of India ink introduced under the skin. It appears that this spreading factor, which is also found in the toxins of various bacilli, is none other than the enzyme hyaluronidase. It is well known that the fluid in the interstitial spaces of animal tissues combines with hyaluronic acid which belongs

TABLE 1

Statistical data

Distribution of cases according to:

1. *Sex.* Male—44; female—17

2. *Age in years*

0– 9	4
10–19	17
20–29	18
30–39	13
40–49	6
50–59	2
Over 60	1

3. *Month of occurrence*

January	1
February	1
March	1
April	2
May	5
June	9
July	12
August	10
September	12
October	7
November	0
December	1

4. *Degree of seriousness*

Light	26	
Medium	14	
Severe	4	
Critical	13	
Fatal	4	(all between 1940–1944)

5. *Interval in hours between bite and hospital admission*

	Cases	Died		Cases	Died
Less than ½ hrs.	1	0	3 to 6 hrs.	5	0
½ to 1 hrs.	6	1	6 to 12 hrs.	5	2
1 to 3 hrs.	40	1	Over 12 hrs.	4	0

6. *Location of bite.* Upper limbs—35; lower limbs—26

7. *Circumstances attending the bite*

The majority of bites (31 cases) occurred during accidental treading on the snake. The next most frequent cause (21 cases) was that of putting the hand into a place which was concealed from sight. Three cases occurred during sleep and two during an attempt to grasp the snake with the hand.

to the mucopolysaccharide acids. This substance unites the cells of the connective tissue with the aid of a jellylike matrix and is itself broken up under the influence of hyaluronidase, thereby increasing the permeability of the tissues. The venom passes from the interstitial tissues to the lymph channels and thence, indirectly, into the circulation of the blood. Experimental proof for this view is also found in the works of Barness and Trueta (1941). These authors have again

confirmed that substances of low molecular weight are absorbed through the lymph vessels. They have shown that viper venom, whose molecular weight is greater than 20,000, is not absorbed from the tip of the toe of a rabbit if the lymph vessels are cut. However, if these vessels remain intact but the blood vessels between the upper and lower leg are cut, then the venom is absorbed with ease. Cobra venom, whose molecular weight is less than 5,000, is absorbed through the blood vessels even though the lymph vessels are cut. These facts

TABLE 2

Clinical manifestations of viper bite in their order of frequency in 61 cases

Local swelling	58
Local pains	56
Vomiting	36
Discoloration (skin)	33
Shock	26
Fever	26
Diarrhea (bloody stools in 6)	20
Anemia	20
Abdominal pains	19
Sanguinolent blebs	17
Serous blebs	13
Quincke's edema	15
Toxic lymphangitis	11
Nausea	11
Weakness	11
Cold perspiration	7
Retention of urine	5
Skin hemorrhages (except the region of bite)	5
Restlessness	4
Bradycardia	3
Hemorrhages in the mucous membranes	3
Disturbances in swallowing	2
Hematuria	1
Late sequelae	
Lymphedema	3
Contracture (of Dupuytren's type)	2
Neuritis, peripheral	1
Gangrene of foot	1

gain special importance in treatment, for the flow of lymph (and of course the absorption of venom) is dependant on the movements of the muscles of the limbs. Active movements increase the flow of lymph, wheras complete rest of the muscles stops the flow of the lymph entirely.

THE CLINICAL PICTURE

Table 2 summarizes the clinical signs of snake bite in their order of frequency; 61 cases were investigated from this aspect. Table 3 shows their patho-physiological relations. Four typical cases of snake bite are described below.

Case No. 25 (Chart I, Fig. 1)

A. K., male, aged 24, was admitted to hospital on May 10, 1945. About ½ hour before this he was out walking, dressed in sandals, and was bitten by a snake. The snake itself he did not see. He was immediately taken to the local clinic. There his leg was bandaged above the knee and after the bites had been somewhat enlarged by a scalpel, crystals of potassium permanganate were rubbed in. He was also given an injection of Serum Antivenimeux prepared in South Africa. Several minutes after the bite he became pale, began to vomit and to have diarrhea which was accompanied by abdominal pain. Immediately after this he felt his tongue swelling and he was covered with a cold sweat.

On admission it was noticed that his tongue was swollen to two or three times its normal size. His lips were also considerably swollen. The pulse was impalpable. Blood pressure was 60/45 mm. Local findings: on the outer and anterior part of the dorsum of the right foot were small scratches stained violet, with swelling around them. There was severe hemoconcen-

TABLE 3

Clinical manifestations according to their patho-physiological relations

I. *Anaphylactoid syndrome (association of vomiting, diarrhea, shock, etc.)*

	Cases		Cases
a) Anaphylactoid shock: mild	5	d) Eosinophilia early	9
moderate	8	late	8
severe	12	after administration of serum	12
b) Quincke's edema	15	e) Disturbances in swallowing (edema?)	2
c) Urticaria	2	f) Thrombocytopenia	2

II. *Local signs*		III. *Hemorrhages*	
Swelling	58	Skin, local	26
Discoloration	33	Skin, distant	5
Cutaneous blebs	30	Melena	2
Sanguinolent	17	Hematemesis	2
Serous	13	Hemorrhages in mucous membrane	3
Traces of the snake's denture	34	Epistaxis	2
Typical	8	Hematuria	1
Non-typical	26	Anemia	20
Toxic lymphangitis	11		

tration: r.b.c. 5,6 million, w.b.c. 17,000, Hb. 17 gm %. Urine: albumen +, urobilinogen +, sugar −, deposit −.

12 midnight. The diarrhea had ceased, the patient was still vomiting and the tongue was still swollen. The pulse was now palpable, soft. Patient very dry. A drip transfusion of physiological saline was started. B.P. 90/60. Respirations slow and deep.

1.30 a.m. Still vomiting. B.P. 100/65. Transfusion continued and supplemented with blood. The dorsum of the right foot near the ankle was swollen and the skin in this area was bluish and tender to pressure.

8.30 a.m. Feels well. B.P. 125/80. The drip has been continued all night and during this time he has received, in addition to a solution of salt and glucose, a liter of fresh, citrated blood. The tongue is now only slightly swollen. The right calf swollen to above the knee-joint. The foot now blue. Transfusion stopped. Urine: albumen +, urobilinogen −, sugar −, deposit nil.

5 p.m. General condition good. Complains of pain in the right arm and leg. The thigh swollen to the inguinal region. Extensive subcutaneous hemorrhages in the medial part of

the right thigh. Further hemorrhages noticed in the groins. The skin of the right forearm swollen and shows purpuric spots. The tongue now normal in size.

Restless and vomiting during the evening. B. P. 100/70-130/30.

May 12, 9 a.m. The swelling has now risen above the groins and the difference in the circumferences of the thighs is 14.5 cm. Extensive hemorrhages in the skin, and on both sides of the calf there have appeared large blisters filled with serum (see Fig. 1).

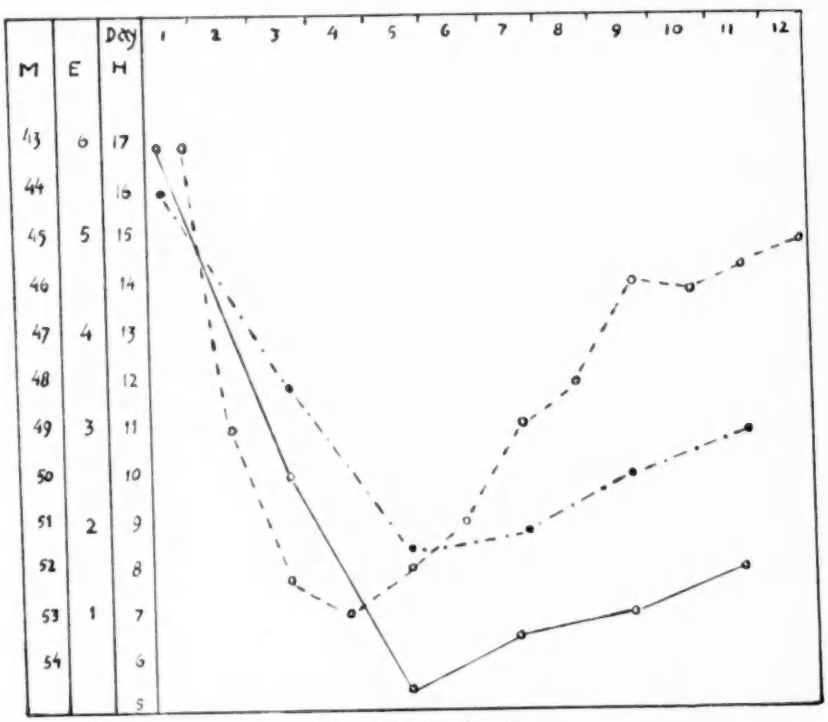

CHART I. *Case No. 25*

Legends

M Average of Combined Measures

E . . . . . Red Blood Corpuscles

H Hemoglobin

5 p.m. Nausea and vomiting several times. Progressive swelling in the right lumbar region with extensive areas of hemorrhages under the skin.

May 14. Progressive pallor. Blood count shows severe anemia: r.b.c. 1.8 million, Hb. 5.5 gm %, w.b.c. 6,000, icterus index 4, platelets and reticulocytes normal. Swelling is now up to the lower border of the ribs together with widespread hemorrhages. In the mucous membrane of the palate numerous hemorrhagic spots and small white areas. Swelling of the thigh diminishing. Several of the blisters have now burst and are oozing serum. The hemorrhagic areas are turning yellow. A further blood transfusion is given: 250 cc. packed red cells and $\frac{1}{2}$ liter fresh, citrated blood. Circulation normal.

May 16. Still pale. Blood count: r.b.c. 1.8 million, w.b.c. 6,500, reticulocytes 6%, eosino

philes 15%. Examination of the fluid from one of the blisters showed: color yellowish, albumen 4.2%, red and white corpuscles in the same proportions as in the blood. Van den Bergh, direct and indirect, negative. Nothing abnormal detected in the urine. Complains of headache. B.P. 120/70. During the last two days has been excreting between 2 and 3 liters of urine in the 24 hours.

May 20. General condition excellent. Rapid diminution of the swelling. Blood count: r.b.c. 2.8 million, w.b.c. 7,000 (eos. 5%), reticulocytes 6.6%. Got up. No abnormal findings in the urine. Specific gravity 1018.

FIG. 1

May 28. Discharged. Condition good. The very extensive swelling has almost completely disappeared. Only the skin is stained bluish yellow. Blood count: r.b.c. 4.3 million, Hb. 11 gm %.

Case No. 44 (Chart II)

R. B., female, aged 46, was admitted to hospital on July 26, 1946, at 11.15 p.m. At 9.30 p.m., while walking in the grounds of the settlement, saw something shining on her legs and suddenly felt a stabbing sensation on the dorsum of the right foot. She proceeded a little further and then a doctor was brought to her. He put her foot in a splint and put ice on it. She was then sent to hospital on a lorry. The ride was a very rough one and each bump gave her a pain in her abdomen. About ½ hour after the bite she started to vomit and to have diarrhea. The diarrhea was continuous and accompanied by severe abdominal pains. Passed urine together with feces. Arrived in hospital at 11.15 p.m.

On admission was fully conscious, the skin was covered with a cold sweat, the pulse was impalpable and blood pressure could not be read. Continual diarrhea. At midnight a drip was started. Locally the right leg was swollen. In the middle of the dorsum, close to the internal border, were visible bluish spots on the skin, two of which were much larger and separated from each other by 1 cm. Round these spots there was a blue discoloration.

Examination of the blood showed hemoconcentration: r.b.c. 5.73 million, Hb. 19 gm, w.b.c. 17,000 (polymorphs 78%, stab 14%, lymphos 4%, eos 1%). In testing for the coagulation time the blood clotted in a dry test tube after four minutes but after an hour became fluid once again (fibrinolysis?).

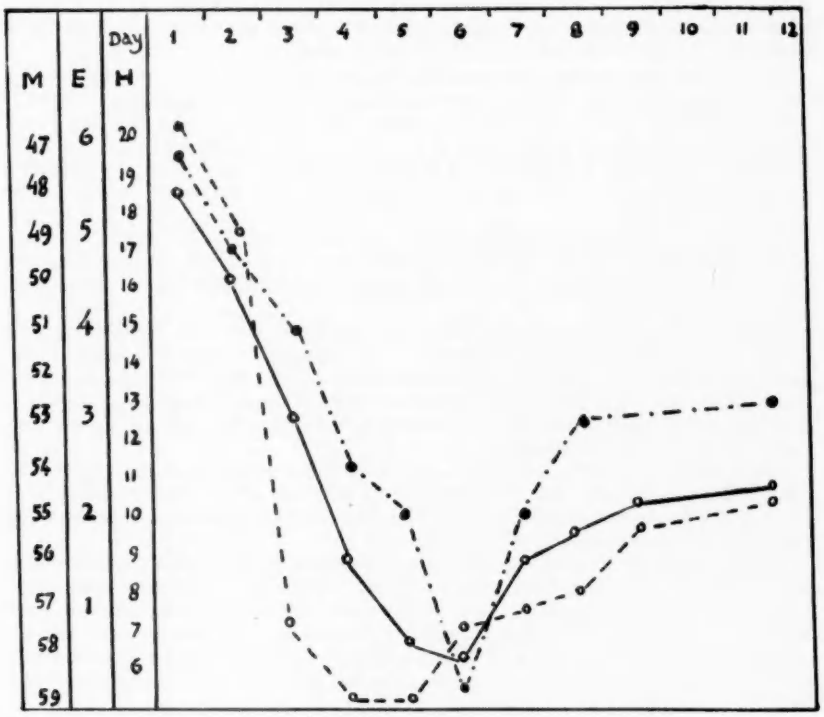

CHART II. *Case No. 44*
Legends as in Chart 1

Progress of the illness (see also Chart II)

July 27. 1.45 a.m. B.P. 80/60. Feeling much better but continues to have diarrhea and to vomit. Thirsty.

3 a.m. Diarrhea and vomiting. B.P. 75/50. Pulse rate 120 per minute. Bladder catheterized and 600 cc. of urine removed. Urine showed albumen +, sugar and urobilinogen absent, and in the deposit, hyaline and granular casts.

12 noon. B.P. 75/50. Extensive urticaria on both sides of the abdomen. Taking fluids freely. Drowsy.

5 p.m. Slight collapse. The B.P. fell to 60 mm. Hg. systolic despite the continuing drip transfusion. Given a transfusion of 250 cc. of blood and a further liter during the course of the day. Several ampoules of ephedrine were added to the solution of glucose and saline which was being given by the drip.

8 p.m. Weak and drowsy. Temperature rise to 38°C.

12 midnight. Vomiting, diarrhea, profuse sweating. The swelling of the foot increased

during the day and spread to above the knee. Numerous widespread hemorrhages on the foot.

July 28. The B.P. during the day varied between 95/65 and 105/70. The patient continued to vomit and to have diarrhea but less than the day before. The drip was continued and a further transfusion of 250 cc. of blood was given. The temperature still up and reached 39°C. Passed 850 cc. of urine in the 24 hours. The swelling now above the pelvis. Widespread hemorrhages on the dorsum of the foot, the posterior part of the calf and on the inside of the thigh. During the day the skin showed a bluish marbling.

July 29. Feels much better. Drinking freely and passing urine in large quantities. Specific gravity of urine 1010. The swelling reaches to the abdomen and lumbar regions and spreads over to the vulva and the left groin. The labia majora very swollen and distended with blood. Large hemorrhages in the region of the groins. Blisters on the leg and the calf. The transfusion continued. The patient has become pale. Blood examination showed severe anemia: r.b.c. 2.75 million, Hb. 8 gm %, w.b.c. 14,000, the differential count showing a shift to the left.

July 30. The improvement continues. The drip discontinued. Hemorrhages in the skin of the pelvic region and the lower legs, especially in areas subject to pressure. The B.P. rose to 120/80. Urine: albumen +, and in the deposit granular casts and leucocytes; glucose and urobilinogen absent.

July 31. Pallor still more marked. The blood picture showed r.b.c. 1.2 million, Hb. 6 gm %, w.b.c. 7,200, occasional reticulocytes only. The differential count showed polymorphs 64%, stabs 2%, lymphos 30%, monos 3%, eos 1%. Plasma bilirubin 1.1 mg %. Given 600 cc. blood and 800 cc. of blood cells freed from plasma. During the transfusion complained of difficulty in breathing. The blisters on the swollen leg became so big that they had to be emptied by puncture.

Aug. 1. Spread of swelling halted. The swollen leg shows numerous yellowish and bluish blisters. The sound leg shows thrombophlebitis where the transfusion was given. Still complaining of difficulty in breathing. Dullness over the lower part of the back of the right lung and diminished breath sounds. B.P. 125/70.

Aug. 3. Feels well but weak. Breathing free. The swelling diminishing. The hemorrhage has spread over nearly the whole of the lower body. Numerous blisters on the back of the foot and the calf, some closed and some with a serous discharge. B.P. 130/70. After some improvement in the diarrhea during the last two days, started once again to pass numerous motions. No parasites were found in the stools. Urine: albumen +, urobilinogen +, and in the deposit, leucocytes. Temperature during the last few days has kept in the region of 38°C.

Aug. 4. Blood: r.b.c. 2.3 million, Hb. 8.5 gm %. w.b.c. 16,500, reticulocytes 16.5% (!) Polychromatophilia. Anisocytosis. Bilirubin 1.5 mg %.

Aug. 8. General condition good. Decrease in the swelling maintained. The hemorrhagic areas becoming yellow. The blood still shows reticulocytosis. The looseness of the bowels improved. Anesthesia of the toes and the foot that has been bitten, but normal sensation in the lower calf.

Aug. 19. Feels well. Diarrhea stopped completely. Passing urine freely. The specific gravity has risen to 1020. Sensation in the skin of the toes still absent. An ulcer covered with a black crust has appeared on the dorsum of the bitten foot. Under the crust there is a purulent discharge. Blood picture improved. r.b.c. 3 million, Hb. 12 gm %, w.b.c. 7,600. Reticulocytes have fallen to normal. The swelling of the bitten extremity almost completely disappeared.

Sep. 1. General condition very good indeed. On the back of the bitten foot an ulcer 3 x 5 cm. with good granulations at its base.

Sep. 25. Up and about. No abnormal findings in the urine.

Follow up on Jan. 2, 1947.

The wound on the right leg completely healed. Feeling of tension in the toes as if the foot was "in a vise". Tires on walking and the right foot "drags". The skin still bluish.

The pulse in the dorsalis pedis easily felt. Scars on the dorsum of the foot. Toes turned upwards except for the big toe. Absence of sensation in the skin of the toes.

Case No. 26 (Chart III)

M. M., aged 17, female. Arrived at the hospital at 6 in the evening on May 26, 1945. She had slept on the seashore of the Sea of Galilee and at dawn felt cold. Put her hand into the rucksack to extract a blanket. In the rucksack there was a snake which bit her on the tip

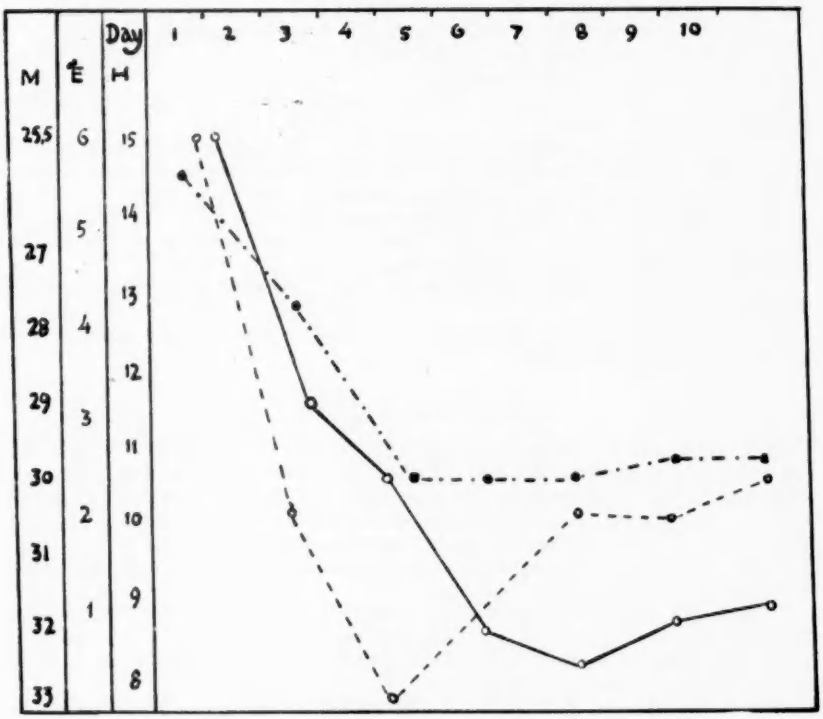

CHART III. *Case No. 26*
Legends as in Chart 1

of the middle finger of the right hand. Several minutes later her tongue swelled up and she found it difficult to speak. Ten minutes later she received first aid treatment, and was given an injection of Serum Antivenimeux. Her companions bound up her hand immediately after the bit ' ut later removed the bandage. About ½ hour after the bite she started to vomit and to have diarrhea. On admission complained of abdominal pain. She was menstruating and in the second day of her period.

Condition on admission. Fully conscious, restless and throwing herself from side to side. Diarrhea. Vomiting from time to time. Pulse not palpable. The B.P. could not be measured. Hemoconcentration (r.b.c. 6.4 million, Hb. 15 gm %, w.b.c. 33,800). Incised wound on tip of right middle finger and the whole of the tip blue. Tongue very swollen and about 2 to 3 times its normal size. Lips also swollen. Tachycardia—116 per minute—regular.

7.30 a.m. Covered with a cold sweat.

9.30 a.m. Marked acrocyanosis. Vomited at times. Deep sighing respirations. Covered with a cold sweat. Veins collapsed. Entry into a vein in order to give a drip transfusion was successful only two hours after admission.

10.30 a.m. Right hand swelling. Hemorrhages on the inner side of right upper arm.

2 p.m. Pulse now felt. So far has not passed urine. Receiving transfusion of blood and plasma.

4 p.m. Feels more comfortable. Drinking freely. Vomiting ceased. Only one attack of diarrhea. Pulse very rapid indeed.

10 p.m. Diarrhea with bloody stools. Tongue now back to its normal size.

Midnight. Feels well. Continues to pass bloody stools.

May 27. Hematuria. Right arm swollen.

May 28. Feels well. Blue spots on tongue. Good urinary output. Urine concentration normal. Transfusion stopped.

May 29. Pale. Blood count: r.b.c. 2.8 million, Hb. 8.5 gm %. w.b.c. 7,500, occasional reticulocytes. Urobilinogen not detectable in the urine. Ecchymosis on the hard palate. Tongue still blue. Numerous hemorrhages in the right armpit.

June 4. Feels well. Still anemic. Diarrhea started afresh.

June 9. Loose motions continue. Free *E. histolytica* in the stools. Course of emetine injections commenced. Swelling of right arm less.

June 15. Received 6 injections of emetine, 0.05 gm. Diarrhea stopped after the second injection. Up and about.

June 17. Feels well. Urine: no abnormalities. Blood count: r.b.c. 2.9 million Hb. 10 gm %, w.b.c. 5000. Discharged.

Case No. 58 (Chart IV)

M. A., male, aged 10. Admitted on Oct. 28, 1947, at 8.30 p.m. About two hours previously was bitten by a snake on the top of his right foot. His friends saw a large snake slide away. Following the bite he played around for about 3 minutes. Fifteen minutes later he started to vomit, went pale and became pulseless. He was bandaged above the knee and the bandage was removed about 20 minutes later. The leg was put in a splint and ice was packed around it. While being conveyed to the hospital was restless but did not vomit or have diarrhea. Complained of thirst and abdominal pain.

Condition on admission.. Vomited in the receiving room. Pulse soft but not very fast. B.P. (systolic) 60–65 mm. Hg. Tongue and upper lip swollen. Immediately on admission a drip transfusion was started. Hemoconcentration: r.b.c. 5.8 million, Hb. 19 gm %, w.b.c. 40,000. Differential count normal.

10 p.m. Drowsy. B.P. 85/60. So far has not passed urine.

Oct. 29. Slept fitfully during the night and passed urine. The urine contained albumen. The whole leg swollen and the swelling extended to the right lower abdomen. Small blood-blisters appeared on the side of the bite. Large subcutaneous hemorrhages on the upper part of the foot, the inside of the calf and thigh and the groins. Vomited in the forenoon. The vomitus contained blood clots. Platelets 25,000 per cmm. B.P. 110–120/80.

Oct. 30. The swelling extending over the abdominal wall up to the umbilicus. Patient markedly pale. Blood picture: r.b.c. 3.23 million, Hb. 12.7 gm %, w.b.c. 16,000.

Oct. 31. Further drop in r.b.c. (2.8 m) and Hb. (8 gm %). Given transfusion of 1 liter blood. The swelling has reached the right rib border and the left groin. Large subcutaneous hemorrhages in the swollen areas.

Nov. 2. The blisters have opened and ooze serum. The swelling on the right side is up to axilla.

Nov. 4. No further increase in the swelling. The hemorrhagic areas turning yellow. Hemorrhages in the mucous membrane of the eyelids. Platelets 28,000 per cmm. Blood values rising. Feels well. Albumen constantly present in the urine.

Nov. 6. Made a good recovery and left the hospital in good condition. On discharge the blood count showed: r.b.c. 3.8 million, Hb. 13 gm %, w.b.c. 7,000. Albumen absent from urine. On the skin of the dorsum of the foot close to the site of the bite a healing ulcer.

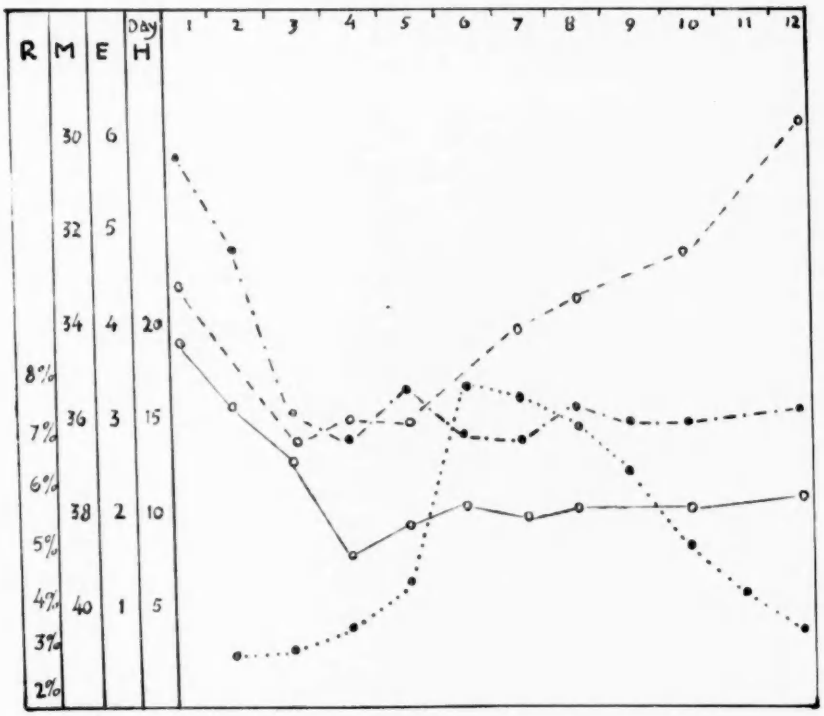

CHART IV
Legends as in Chart 1, plus "R"
R ..... Reticulocytes

PATHOLOGICAL CHANGES

There are few references in the recent medical literature to the pathological changes following snake bite, especially in man, whereas there are a number of articles dealing with the changes in experimental animals. Taube and his co-workers published (1937) their findings on the pathological changes following the injection of rattlesnake venom into dogs, and Fiedler *et al.* (1940) on the resultant changes following the subcutaneous injection of rattlesnake venom into the *Macaca mulatta* monkey.

We have had the opportunity of carrying out examinations in four cases of snake bite.

Case 1. Woman, age 40. Bitten at 9.30 p.m. in two places in the lower lip. Severe collapse occurred very quickly, about 20 minutes later. A doctor extended the wounds and placed

some potassium permanganate crystals into them and gave an injection of Serum Antivenimeux. The patient was expiring by the time she reached hospital some two hours later and despite intensive antishock treatment died shortly afterwards.

Case 2. Male, age 33, bitten by a snake at 2 in the morning on his left thumb. His arm was immediately bandaged above the bite, but the doctor who examined him removed the bandage because he failed to find any evidence of a bite. Two hours later he began to have diarrhea with bloody stools and collapsed. He died about 10 hours after the bite.

FIG. 2

Case 3. Male, age 60. Bitten on the foot during the evening and shortly afterwards received Serum Antivenimeux. Developed bloody stools and died about 18 hours after the bite.

Case 4. Girl, age 15. While out walking felt a prick on her leg. (This occurred about 6 hours before her admission to hospital.) Continued walking but 20 minutes later became unconscious. The doctor who examined her suspected a fracture because of the blue and swollen state of her leg. Several hours later there was hemorrhage from the bowel and the patient was admitted to hospital. There the swelling spread and reached to above the groin. The patient died 48 hours later in a state of shock.

As the anatomical and histological changes in all 4 cases were similar, it is possible to describe the findings in all of them together. Outstanding were the swelling and the hemorrhages. The skin, subcutaneous tissue and the muscles

were infiltrated with sanguineous fluid. These changes were also found in areas proximal to the bites as well as in areas distant from them. The regional lymph nodes were swollen and hemorrhagic; e.g., in bites of the leg the inguinal group of lymph nodes were chiefly affected.

Extensive hemorrhages were noted in the internal organs. In all the cases extensive hemorrhages were found under the endocardium of the left ventricle, in

FIG. 3

the septum and papillary muscles. Petechiae were also found in the pleura, peritoneum and in the mucous membrane of the pelvis of the kidney and of the urinary bladder. In two cases hemorrhage into the mucous lining of the stomach and intestine were noted. At times the blood remained fluid in the vessels many hours after death and only clotted while postmortem was performed.

As would be expected from the macroscopic findings the outstanding histologic changes were the hemorrhages and acute stasis. Around the site of the bite was found a transudate of serous blood-stained fluid into the skin, subcutaneous tissue and the muscle. In the area immediately surrounding the bite there was an obvious leucocytic reaction (Fig. 2) as well as marked changes in the walls of the arterioles and capillaries. At times the wall was blurred and the endothelial

cells were swollen. Occasionally there was a tear in the wall with hemorrhage around it. Similar changes were also found in other organs distant from the site of the bite. Fig. 3 shows a small vessel in the pons with a tear and hemorrhage in the perivascular space.

Lymph nodes: great distention of the vessels with hemorrhages (Fig. 4). The endothelial cells of the sinuses are separated and show signs of phagocytosis.

FIG. 4

Kidneys: the blood vessels are distended both in the cortex and medulla. The capillaries of the glomeruli are stuffed with red blood cells (Fig. 5). At times there is an interruption in the wall of the capillaries accompanied by hemorrhage into the lumen of Bowman's capsule.

Intestines: the changes may be very severe. The capillaries of the mucous lining are greatly enlarged and distended with blood (Fig. 6 and 6a).

Lungs: distended and hemorrhagic.

The changes in the parenchymatous organs, except for those in the blood vessels, are few. Only the liver showed a slight proliferation of cells infiltrating into the sinuses which were distended. Fig. 7 shows the separation of the basal

membrane from the cells of the parenchyma (Seröse Entzündung, Eppinger).

Adrenal cortex: in one case fairly heavy lymphocytic infiltration was noted (Fig. 8).

Table 4 gives the laboratory findings in the cases examined.

FIG. 5

PATHOGENESIS

Viper venom, as we have seen, causes first and foremost damage to the blood vessels and especially to the arterioles and capillaries. In them it leads to the escape through the damaged walls of fluid and solid contents of the blood. (Hemorrhage by diapedesis). At times, however, whole portions of the wall disintegrate and this leads to hemorrhage by rhexis.

These changes are found also in animal experiments. As far back as 1886, Reichert and Mitchell expressed the opinion that snake venom has a direct influence on the wall of the vessels. According to Fiedler there is a disintegration of the vessel walls and the connective tissue at the site of the bite which causes an outflow of fluid. At the same time the lymph vessels open up and the venom is carried to the regional lymph nodes. Here very severe changes occur and, as the

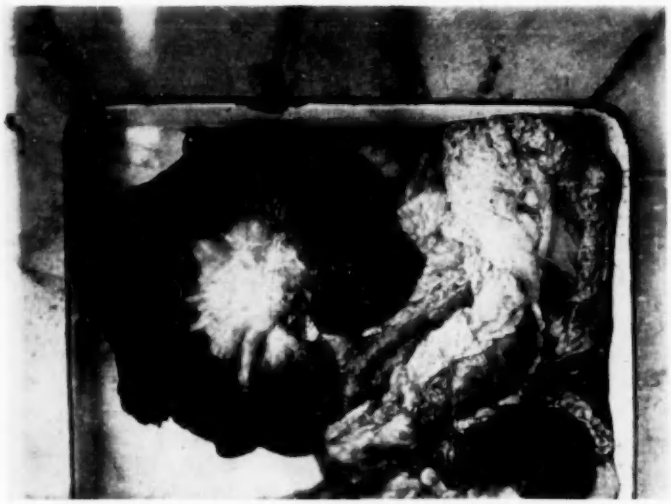

FIG. 6

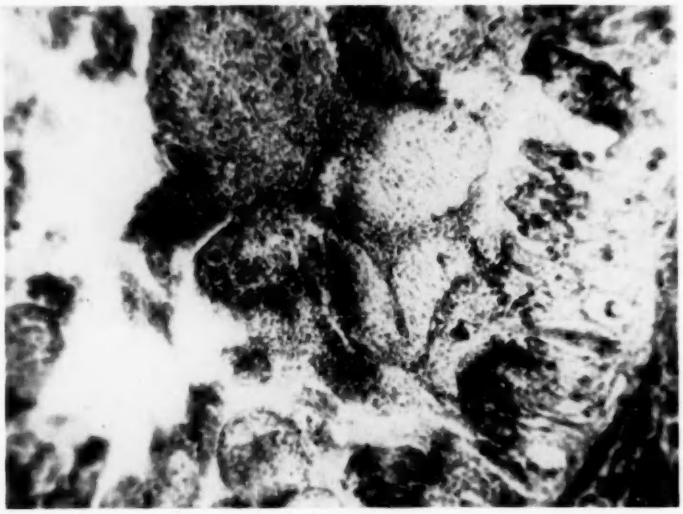

FIG. 6a

venom passes out of these nodes, fresh lymph vessels open up and so the cycle of events is repeated. In the interstitial spaces the venom further damages the arterioles and capillaries.

As has been pointed out above, the hemorrhages extend along the length of

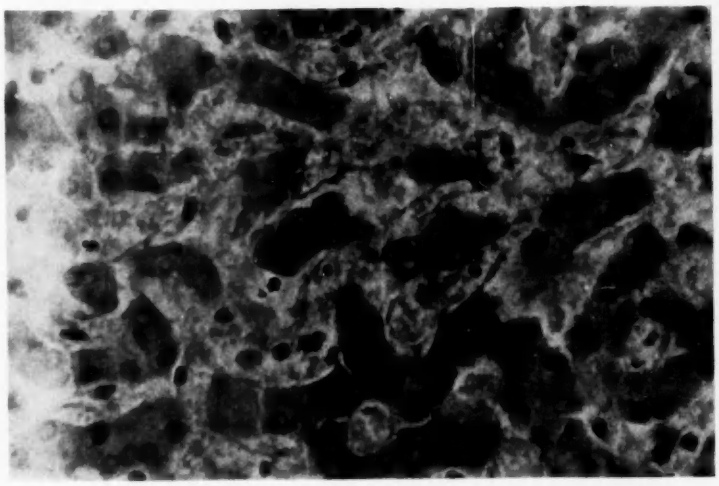

FIG. 7

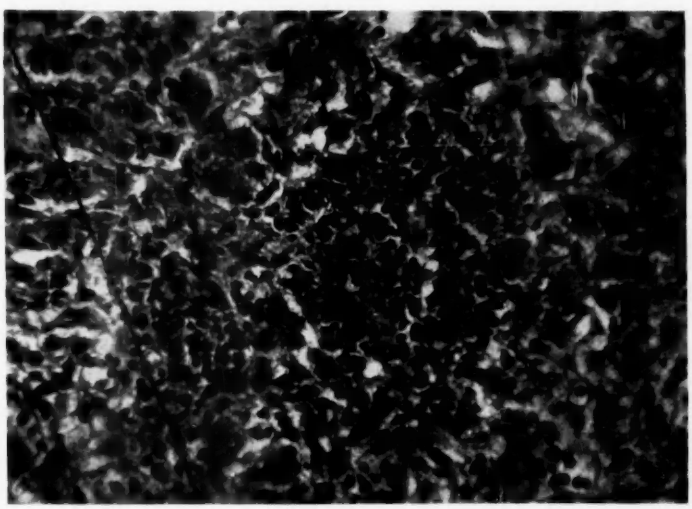

FIG. 8

the lymph channels in affected limbs. In two cases (one only is included in the present series) it was noted that there were hemorrhages in the region of the ramification of the lymph vessels in the serosa of the uterus, and in the second, of the bladder and retroperitoneal tissue. It is noteworthy that these hemorrhages were confined to one side only and did not pass over the midline.

The clinical picture is made up 1) of local signs such as: swelling, subcutaneous hemorrhages, blood and serous blisters, hemorrhage into the soft tissues, and 2) of general signs. The local signs are caused, as has been pointed out above, by the damaging effect of the venom on the capillaries of the blood and lymphatic systems. The venom is absorbed and gives rise to hemorrhages both local and at

TABLE 4

Laboratory findings in cases of snake bite in Israel

I. Hematology

1. *Hemoconcentration on admission*

Number Examined	Present	Absent	Max. Hb.	Max. r.b.c.
39	28	11	22.5 gm%	6.6 m.

2. *Leucocytes on admission*

	Cases
a) Leucocytosis, 10,000–20,000	20
20,000–30,000	8
30,000–40,000	5
40,000	1
b) Leucocytosis in cases without hemoconcentration	11 (range: 12,200 to 24,000)
c) Leucopenia	2

3. *Platelets*

On admission		One week later	
Normal	Decreased	Normal	Decreased
4	2	5	1

	No. exam.	Slow	Normal	Increased
4. Sedimentation rate of r.b.c.	18	14	3	1
5. Coagulation time	13	0	13	0
		Present	Absent	
6. Fibrinolysis*	12	5*	7	

7. Prothrombin time. Prolonged only in cases in a state of shock

II. Urinalysis

	On admission	Subsequently
1. Normal findings		22
2. Albuminuria	16	23
3. Urobilinogenuria	6	24
4. Red blood corpuscles	4	6
5. Leucocytes	8	9
6. Granulated casts	9	10

* All in shock.

a distance and brings into operation a chain of generalized reactions which take on the form, in the first instance, of anaphylactoid shock. It is manifested in the gastro-intestinal system by nausea, vomiting, abdominal pain and incessant diarrhea, in the cardio-vascular system by severe peripheral shock (drop in blood pressure, disappearance of pulse, palpitation, hemoconcentration) and by allergic signs (urticaria, Quinke's edema in 15 cases, and eosinophilia).

This symptom complex at the onset has a remarkable resemblance to the

picture of anaphylactoid shock in man as described by Urbach and Gottlieb: "The clinical picture is striking; vomiting and violent colonic spasms appear, causing unbearable abdominal pain and uncontrollable diarrhea. There is an abrupt fall in the blood pressure, accompanied by a drop in temperature (although in occasional cases the temperature will rise). The patient becomes strikingly pale and finally cyanotic. Tachycardia is very commonly observed: this can be so severe that the pulse becomes imperceptible and the heart sounds barely audible. Acute pulmonary emphysema may ensue. The general condition becomes progressively worse and the patient loses consciousness."

We found no signs which referred to the respiratory system in our cases. In experimental medicine we also find support for the anaphylactoid factor in cases of snake poisoning. P. Boquet quotes Arthus who compared the symptoms following poisoning by snake venom to "Choc sero-anaphylactique". Rocha and Silva (1939–40) also compare the effects of snake poison with that of trypsin, both of which give rise to signs of anaphylactic shock.

Feldberg (1939) and Kellaway and Trethewie (1939) showed that perfusion of animal tissues with venom of various snakes give rise to the production of histamine in the perfusion fluid and protein substances which have the property of clotting (according to Esses, 1945).

This anaphylactoid shock passes readily over to the usual peripheral shock, apparently as a result of the increased outpouring of blood and plasma into the tissues. The typical picture of shock appears—low blood pressure which at times cannot even be measured, soft rapid pulse which sometimes can hardly be felt, and hemoconcentration. We consider the fibrinolysis, which we noted as occurring in a state of shock only, and also the lengthening of the prothrombin time, as due entirely to the shock itself.

The mechanism which brings about the state of shock leads also to its perpetuation. The increased permeability of the blood vessels, and the transudation of blood and plasma resulting from it, bring about the severe anemia which appears in the subsequent days. In all our cases which suffered from shock and severe anemia, we noticed extensive swelling in the form of massive edema which spread centripetally from the limb which was bitten to the body, half of which at times was affected. According to Noble and Gregersen (1946), accumulation of a liter of blood in the thigh increases its diameter by one cm. In several cases we obtained figures very much greater than this as is shown below.

Case no. 21: died of shock in less than 24 hours. The difference in circumference between the thighs was 8.5 cm., i.e. a difference in the diameter of 2.7 cm. In other words 2.7 liters of fluid passed out into the tissues in less than 24 hours.

Case no. 25: peripheral shock lasting 24 hours. Anemia developed over 7 days. On admission the Hb. was 17 gm % and the red blood cells 5.5 m. per cmm. In the course of a week the Hb. fell to 6.5 gm % and the r.b.c. to 1.84 million. The difference in the circumference of thighs was 13 cm. The increase in the diameter was therefore 4 cm in a week.

Case no. 44: shock and anemia. On admission, Hb. 19 gm % which fell to 6 gm % and r.b.c. 5.7 m. which fell to 1.2 m. per cmm. Difference in diameter of thighs was 4.4 cm. (in circumference 14 cm.).

Case no. 27: shock and anemia. On admission Hb. 17 gm % which fell to 10 gm % and

r.b.c. 6.95 m. and later 3.2 m. per cmm. The difference in circumference of thighs was 17 cm., i.e. 5.4 cm. in diameter.

Case no. 58: shock and anemia. On admission Hb. 19 gm % which fell to 10 gm %. r.b.c. 5.8 m. fell to 2.8 m. per cmm. Difference in circumference of thighs 8 cm. and in diameter, 2.5 cm.

These measurements are naturally only possible in the lower limbs because in the upper limbs the swelling very quickly reaches the back and chest, and the calculation is therefore very much more complicated. Two facts must also be taken into consideration: 1) all severe cases received intravenous saline and plasma. 2) The capillaries in the bitten limb are more permeable and there is a greater than normal transudation of fluid through them. Nevertheless we can give credit for a substantial part of the increase of thigh diameter to transudation of the blood. This "exsanguination" also explains the perpetuation of peripheral shock and the severe anemia. In the graphs accompanying the description of the cases we have attempted to show this relation by charting the increase of the circumference of the bitten limb and the variation in the Hb. and r.b.c. In none of our cases did we find signs of hemolysis. The qualitative examination of urobilinogen in the urine was normal; there was no reticulocytosis and the bilirubin in the blood was not increased. Signs of hemolysis appeared only at the end of the week due, apparently, to the breakdown of the Hb. of the blood which had collected in the tissues.

The extent of the anemia is shown by the observations that follow. On admission, 28 cases showed signs of hemoconcentration due to shock. The highest figures we obtained were 7.6 million red blood corpuscles per cmm. and for Hb., 22.5 gm%. As for the state of shock, in 17 cases it was impossible to measure the blood pressure and in a further 9 cases the systolic pressure was less than 80 mm. Hg. The pulse was not palpable in 13 cases and in a further 11 cases it was more than 120 per minute. In the subsequent days, up to the end of the first week, anemia developed in 20 cases; lowest values of r.b.c. were 1.2 million per cmm. and Hb. 3.0 gm%. Despite repeated transfusions which were given in 17 cases in the first 24 hours and which consisted at times of more than a liter of fresh citrated blood, severe anemia developed as we have pointed out above. Spontaneous regeneration, which quickly increased in rate, started in the second week and was associated with a rise in reticulocytes.

TREATMENT OF SNAKE BITE

Recent findings on the absorption of snake venom and the pathogenesis of the symptomatology of the intoxication has had its effect on treatment. The traditional treatment seemed to us outdated and lacking a logical basis. In 1947 we started on a new system of treatment which was subsequently used in the Jordan Valley and in the Galilee. The results of the treatment by the old methods are shown in Table 5.

Before passing on to the consideration of our new system of treatment we will consider the bases of the previous treatment which we have now given up entirely.

1. *Compression of arteries by tourniquet.* As far back as 1938 the Russian authors Schreiber and Maljugina pointed out that the application of a tourniquet does not prevent the absorption of the venom, for the local signs appear above the tourniquet. It also does not prevent the onset of generalized signs. Allen (1939) was also doubtful of the value of arterial compression in preventing the absorption of the venom. He was of the opinion that the venom spreads with great rapidity over a large area simultaneously, apparently via the lymph vessels. It is therefore unlikely to expect any effect from a tourniquet. Moreover,

TABLE 5

Evaluation of methods of treatment of snake bite in Israel

METHOD	COURSE OF THE CASES						
	Total	Mild	Moderate	Severe	Fatal	Shock developed	Swelling advanced
With tourniquet	34	13	9	11	1	14	27
Without tourniquet	23	12	4	6	1	6	14
With serum "Antivenimeux"						Serum sickness	
For prophylaxis	24	8	7	7	2	4	
For treatment	20	8	2	6	3		
Without serum	22	13	3	6	0		
Summary							
"Old line" treatment (tourniquet, serum, transfusions)	49	19	11	15	4		
"New line" treatment (splint, rest, transsions)	14	8	3	3	0		

in addition to the damage caused by the venom itself, this is likely to interfere with the vitality of the bitten limb. Our experience has also shown that the application of a tourniquet which has been in place for some time, leads occasionally to a worsening of the clinical condition, possibly because of the sudden release of a large quantity of venom into the circulation. In view of the slow absorption and of the successful preventive methods, we have entirely done away with the compression of the vessels and we do not even recommend it as a first aid measure.

2. *Excision.* There is no question that early excision of the part bitten could prevent the absorption of the poison. Practically speaking, however, even under the most favorable conditions, the doctor does not see the victim until 15–30 minutes have elapsed and in the meanwhile the venom has succeeded in passing into the interstitial spaces and into the abundant lymph capillaries. The quantity thus absorbed suffices to bring about the local and general effects of poisoning.

3. *Incision and suction.* Some advise that the skin around the bite be deeply

incised and the wound sucked by the mouth or some mechanical apparatus so as to extract the blood and the venom before it is absorbed. There is little doubt that this treatment can help to remove a certain amount of the venom from the area of the bite, but here also we cannot expect any great effect unless it is done within a very few minutes of the event.

4. *Introduction of various substances into the wound and incision.* We have given up this type of treatment because of the lack of efficacy and because of the local damaging effects of some of the substances used, e.g. potassium permanganate. The only acceptable local treatment which we would take into consideration is the injection of a substantial dose of a specific "serum antivenimeux" into the tissues around the actual site of the bite. It is possible that a specific serum has the ability to neutralize the enzyme hyaluronidase and so to interfere with the absorption of the poison.

The principles of treatment which we have laid down for ourselves are divided into 1) first aid measures and 2) medical treatment.

1. First aid measures

a) First and foremost, in our opinion, should be absolute immobilization of the bitten limb by a splint as would be done in the case of an open fracture. Following the animal experiments of Barness and Trueta (1941) we think that this step diminishes to a very large extent the absorption of the poison. The above authors are too far-reaching in demanding a closed plaster which in their opinion is likely to prevent the onset of extensive swelling. We tried their method in two of our cases which had been bitten on the toes without, however, incising the wounds as they recommend. In the first case we succeeded in preventing the onset of secondary shock but we were compelled to split the plaster lengthwise after several hours because of severe pain. The leg, as usual, swelled up during the next few hours but shock did not develop. In the second case the pain was not so severe, due apparently to the exhibition of analgesics, but here again severe interference of the circulation in the limb developed after a few hours. Although in this latter case we split the plaster cast lengthwise we could not prevent the onset of gangrene and a midcalf amputation had to be performed. Shock did not develop. After this sad experience we gave up using plaster casts and instead used plaster "longettes" moulded to the shape of the affected limb.

b) With respect to injection of specific snake venom anti-serum, we did not have any specific antiserum for the snakes of this country. The ones we used were produced by the Pasteur Institute of Algiers, Fitz-Simon in South Africa and by the Pasteur Institute of Paris, but were without effect whatsoever on the progress of the case when given either before the signs of poisoning appeared or subsequently. Table 5 shows this very clearly. In point of fact all the four fatal cases had received serum, whereas those cases which did not receive serum survived. (These four cases are not included in the anatomo-pathologic study but are included in the clinical study. The cause of death in the above four cases was peripheral shock.) In view of the small number of cases it is obvious that no conclusions can be drawn against the use of specific serum. We would be inter-

ested in trying out a specific serum if it should become available. Large doses would be injected subcutaneously in and around the bite, and in severe cases intravenously. However, as long as no specific sera for the snakes of this country are available, we do not intend to use serum for our cases.

c) In view of the fact that during the first stages of the syndrome there are signs of anaphylactoid shock it is reasonable to give antihistamines. These can also be given prophylactically even before the appearance of signs of poisoning. Our own experience in this field is as yet very small but in the few cases where we used these drugs, signs of generalized poisoning did not develop. However, at the moment, no firm conclusions can be drawn from our results. A further point in support of our recommendation is that in the literature it has been shown that the antihistamines neutralize the action of hyaluronidase. Trethewie and Day (1948) tried out Neo-antergan in mice experimentally poisoned by the venom of *Pseudoechesis porphyriacus* and in their opinion this drug is able to prevent the effects of the poisoning. The above snake belongs to the family of Colubridae and one cannot transfer the conclusions reached to cases of viper bite without further confirmation.

d) Speedy removal to hospital, which should be alerted beforehand, and the provision of blood donors are all important. We have insisted on carrying out the above instructions strictly, and despite the primitive state of transportation in this region we have managed to cut down delays to a minimum. To this we ascribe, in great part, our success in treatment; in the last few years there has not been one death in our institution from snake bite although the cases we received were not less serious than those admitted in previous years.

e) As can be seen from the foregoing statements, we have given up all local treatment except for the immobilization of the limb in a splint.

2. *Treatment in hospital*

a) We advise the injection of *specific* anti-serum in sufficient quantities and by various routes.

b) Antihistamines are given and continued as long as the patient is in the anaphylactoid stage. In one of our last cases (not included in the present series) we gave 5 mg. of Bezantine intravenously (a local product similar to Benadryl) because the patient vomited several times soon after the bite and we were unable to give the drug by mouth. Soon after the injection the patient stopped vomiting and further signs of generalized poisoning did not develop. The local signs were also very mild; the bite was on the hand and, apart from swelling which in the days following reached the shoulder, there were no other local signs.

c) No local treatment is given except that of keeping the affected limb in absolute rest by splinting, plaster "longettes" or sand-bags. We did, indeed, in a few cases place ice on the bitten area but we saw no advantage in the progress of the case.

d) The most important part of the treatment in hospital is *combating the shock* by transfusions of physiologic saline, plasma and blood. We also give blood transfusions in hemoconcentration due to shock, because later on, at times

within several hours, there is exsanguination into the tissues. There is no difference whatsoever between the treatment of shock due to snake bite and that of hemorrhage. Table 5 summarizes the results of the treatment and shows the comparison between the old and new systems.

e) There are certain theoretical reasons for the experimental trial of drugs like ACTH and cortisone in viper bites. These substances increase the retention of fluids in the tissues and can therefore be useful in the oligemia which is found in shock. They also dampen the action of hyaluronidase and there is, moveover, some effect of capillary permeability. It is worth-while emphasizing the effect of these hormones in allergic-anaphylactic conditions. Despite these advantages we have so far had no opportunity of trying out treatment on these lines.

SUMMARY

A study of 65 cases of viper bite in Israel is presented; 61 cases from the north of the country were given full clinical investigation and 4 cases from the center of the country were investigated anatomo-pathologically only. The clinical appearances are summarized according to frequency and classified patho-physiologically. Four cases are described in detail. Anatomo-histologic investigations are described and accompanied by photographs. New observations are made on the pathogenesis of the signs and symptoms of the poisoning. Principles of first aid and medical treatment are laid down on the basis of 10 years of clinical experience and a critical study of traditional methods is made. New methods of treatment are suggested for clinical trial.

REFERENCES

ALLEN, F. M., 1939. Observations on local measures in the treatment of snake bite, *Am. Jour. Trop. Med.* **19:** 393.

BARNESS, J. M., AND TRUETA, I., 1941. Absorption of bacteria, toxins and snake venoms from the tissues, *Lancet* **1:** 623.

BOQUET, P., 1948. *Venins de Serpents et Antivenins*, Ed. med. Flammarion.

DURAN-REYNALS, F., 1939. A spreading factor in certain snake venoms and its relation to their mode of action, *Jour. Exp. Med.* **69:** 69.

ESSEX, H. E., 1945. Certain animal venoms and their physiologic action, *Physiologic Reviews* **25:** 148.

FIEDLER, H. H., GLASGOW, R. G., AND CARMICHAEL, E. B., 1944. Pathological changes produced by the subcutaneous injection of rattlesnake venom (*Crotalus*) into *Macaca mulatta* monkeys, *Am. Jour. Pathol.* **16:** 355.

NOBLE, R. P., AND GREGERSEN, M. J., 1946. Blood volume in clinical shock, *Jour. Clin. Invest.* **28:** 172.

SCHREIBER, M. S., AND MALJUGIN, T. A., 1936. Clinical observations on the problem of snake bite, *Vestnik khirurgii* **47.**

TAUBE, H. N., AND ESSEX, H. E., 1937. Pathologic changes in the tissues of the dog following injections of rattlesnake venom, *Arch. of Path.* **24.**

TRETHEWIE, E. R., AND DAY, A. J., 1948. New therapy of ophidiasis, *Austral. Jour. Exp. Biol. and Med Sci.* **26:** 153.

URBACH-GOTTLIEB, 1946. *Allergy.* Sec. Ed. Grune & Stratton, p. 484.

THE DISTRIBUTION OF *ANOPHELES ALBIMANUS* IN THE CARIBBEAN ISLANDS

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INTRODUCTION

Recent attention directed by Senior-White (1950) to the distribution of the Culicid Tribe Anophelini around the Caribbean Sea, presents an occasion to reconsider several published references to *Anopheles albimanus* Wied. in some of the islands in this area. Extensive observations have now been made in all the islands concerned, and because a reference by one of us (Senevet, 1936) to the occurrence of *albimanus* in Dominica and St. Vincent has been frequently cited both directly and indirectly, opportunity is taken to review this matter in the light of later findings during malaria surveys of several of the smaller controversial British units (Charles 1952).

GEOGRAPHY

The complexity of sovereignties in the Caribbean (or West Indian) Islands and diverse methods of grouping them frequently lead to confusion. The following brief description is therefore set out for purposes of orientation.

The islands form a chain in the Caribbean Sea extending from Cuba in the northwest (immediately below Florida) to Trinidad in the southeast (Fig. 1), and include Curaçao, Aruba and Bonaire off the north Venezuelan coast. They consist of two main groups,

(1) Greater Antilles—comprising the four larger units of Cuba, Jamaica, Puerto Rico and Santo Domingo, the latter divided into the Republic of Haiti and the Dominican Republic.

(2) Lesser Antilles—comprising a large number of smaller units extending from the Virgin Islands immediately east of Puerto Rico, to Curaçao.

The British possessions from the Virgin Islands to Montserrat inclusive are administered as the Leeward Islands; from Dominica to Grenada as the Windward Islands; but neither group includes the French islands of Guadeloupe and Martinique, nor the British ones of Barbados, Trinidad, and Tobago.

Similarly the small Netherland units north of St. Kitts are administered as the Netherlands Leeward Islands, while Curaçao, Aruba and Bonaire off the Venezuelan Coast are grouped as the Netherlands Windward Islands.

ANOPHELES ALBIMANUS IN THE LESSER ANTILLES

Disputed areas. Trinidad—In the course of observations on the bionomics of *Anopheles* in Trinidad, Beattie (1932) recorded the presence of *A. albimanus*,

[1] Chief Officer.

[2] Professor Emeritus.

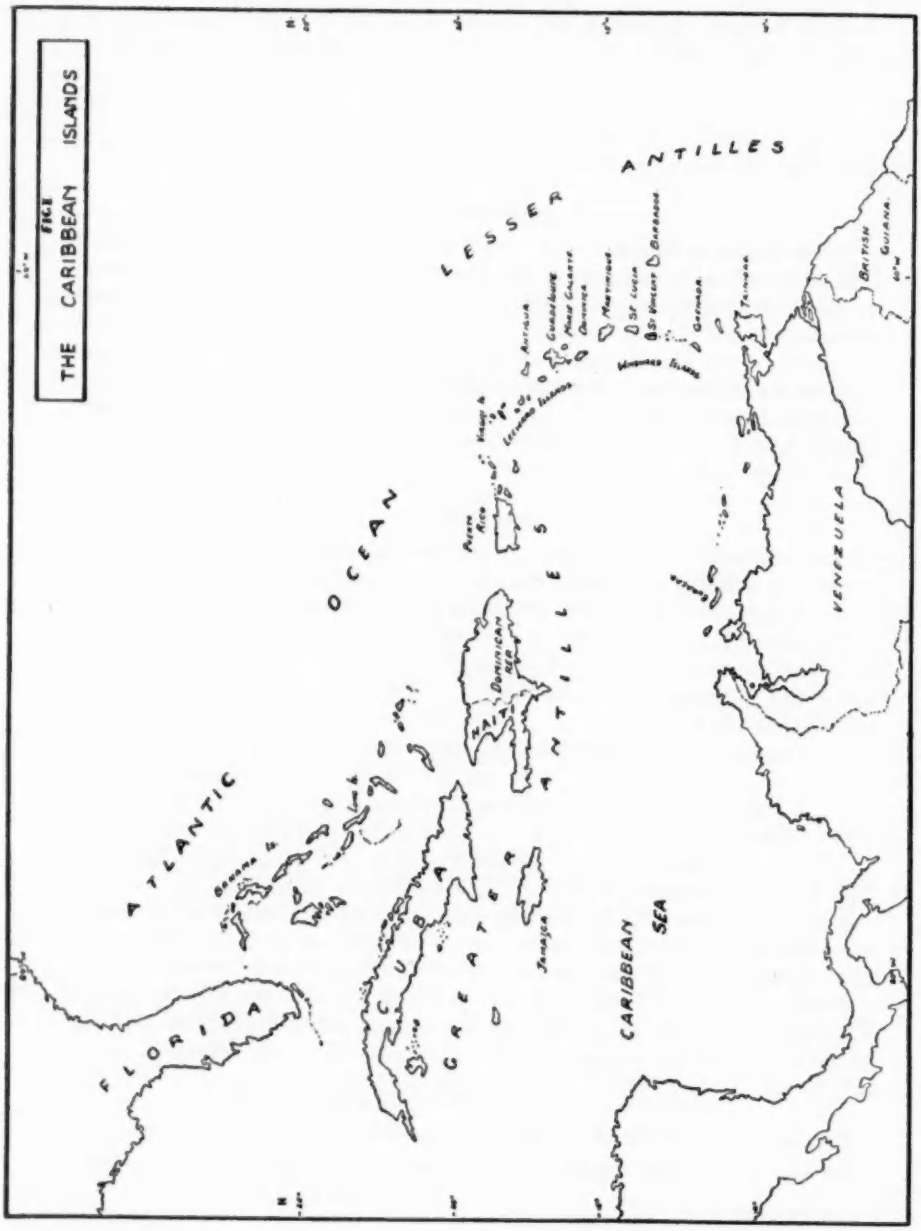

FIG. 1. The Caribbean Islands

but Komp (1937) showed that this record was probably erroneous. Howard, Dyar and Knab (1935) recorded having seen specimens from Trinidad and Grenada. A more recent comprehensive survey by a Rockefeller Foundation team (Downs, Shannon and Gillette, 1943), however, failed to find this species in 467 larval and 247 adult collections made throughout the island.

Grenada—The only reference to this species in Grenada is that of Howard, Dyar and Knab (1935) referred to above, but later field observations by Earle (1936), Root and Andrews (1938), and Charles (1952) produced no specimens although large scale malaria control programs had not been instituted prior to 1942. The last two reports were based on comprehensive malaria surveys of the whole island, with larval collections made in the vicinity of every inhabited locality.

St. Vincent—In an earlier paper, one of us (Senevet 1936) listed St. Vincent as one of the neighboring islands to Martinique where *albimanus* had been previously recorded. This reference was quoted by Rozeboom (1941), who in turn was cited by Simmons and Aitken (1942); and Floch and Abbonenc (1945) used the latter source. Although Senior-White (1950) referred to these French coauthors as his source, he concluded that on the geological evidence available, St. Vincent would be an anomalous locality for *albimanus*.

It is nevertheless of interest to record here that Seagar (1939) reported the presence of this species among specimens submitted from St. Vincent for identification at the Imperial College of Tropical Agriculture, Trinidad, but in an island-wide survey during August–September, 1944, Charles (1952) recorded only *A. aquasalis* and *A. argyritarsis* among 1,428 anopheles larvae.

Barbados—Seagar (1928) investigated a malaria epidemic in October 1927 in this hitherto malaria-free island and had no difficulty in collecting, over a large area, *Anopheles* mosquitoes which were recorded as *A. albimanus*. He considered the possibility of importation by small schooners from British Guiana or "the Antilles."

This source was cited by Rozeboom (1941), and through Simmons and Aitken (1942) by Floch and Abbonenc (1945). Senior-White (1950) noted that although the official report of this Barbados epidemic was attributed to importation of *albimanus* by schooner from Venezuela, importation of *aquasalis*—equally feasible since Charles (1952) found both breeding in artificial containers—had also been mooted. Senior-White reported that the late R. C. Shannon visited Barbados specially during his Trinidad tour of duty but failed to discover any species of *Anopheles* and concluded that whatever the species concerned in the 1927 outbreak, it either was eradicated by control measures or died out naturally.

St. Lucia—Nicholls (1912), reporting on the bionomics and breeding places of *Anopheles* in this island, recorded *A. argyritarsis* and *A. albimanus*, but his identification of the latter was based solely on "the last tarsal joint of the hind leg being almost entirely black"; and on the presence in larvae of "four pairs of brilliant white areas situated on the front of the thorax and on the 2nd, 5th, and 8th abdominal segments." He observed, however, that in many surroundings "these (larval) marks grow indistinct and may entirely disappear."

Although Senevet (1936) also listed *A. albimanus* in St. Lucia, this reference,

unlike that for St. Vincent and Dominica, has not been quoted in the more recent literature. This is presumably due to Earle's (1936) paper which did not record *albimanus* here. A later and comprehensive survey by Charles (1952) confirmed the absence of this species in St. Lucia, for, in an island-wide collection of 1,790 *Anopheles* larvae only *A. aquasalis* and *A. argyritarsis* were identified.

Martinique—During 1934 Senevet (1936), working on this French island, collected only *A. tarsimaculatus* (*aquasalis*) and *A. argyritarsis*. It was nevertheless considered that *albimanus* might possibly occur here, but later observations by Caubet, *et al.* (1948) also yielded only *tarsimaculatus* (*aquasalis*), and *argyritarsis* among 3,325 larvae and pupae collected. *A. albimanus* has not been subsequently demonstrated here.

Dominica—As for St. Vincent, the 1936 publication on Martinique which listed *albimanus* in Dominica has been directly cited by Rozeboom (1941) and indirectly by Simmons and Aitken (1942), Floch and Abbonenc (1945) and Senior-White (1950).

The only record of wide-spread *Anopheles* collections in this island, however, is that of Charles (1952) who failed to identify *albimanus* among an island-wide collection of 2,370 larvae during the favorable period of low rainfall March–May, 1944.

Guadeloupe—François Julien (1930) considered *"Cellia albimana"* as a probable species here. In 1936, *A. albimanus* was collected in the eastern low-lying half of this French island by Senevet (1938), and its presence was confirmed (in both the eastern and western halves of the island) by Floch and Abbonenc (1945). These workers also found it widespread in the small island dependency of Marie Galante southeast of Guadeloupe, in longitude 61°79′ W and latitude 15°53′ N.

Gabaldon (1949) reported that *albimanus* extended only as far south as Guadeloupe, and concluded that it was absent from the Lesser Antilles between the 10th and 15th parallel.

Undisputed areas—North of Guadeloupe, *A. albimanus* was recorded by Hoffman (1930) in Montserrat and Nevis; by Edwards and Box (1940) in Antigua; and Charles (1952) collected it in all three of these islands, but not in St. Kitts.

In the American and British Virgin Islands, Hoffman (1930) constitutes good authority for the presence of this species in St. Croix, St. Thomas and Tortola, while in the Netherlands Leeward and Windward Islands, Van der Kuyp (1949) recorded only one *Anopheles*—*A. pseudopunctipennis*—in Curaçao.

ANOPHELES ALBIMANUS IN THE GREATER ANTILLES

Anopheles albimanus in the four large units of the Greater Antilles (Fig. 1) has been amply confirmed, for example by Earle (1930) in Puerto Rico; Paul & Bellerive (1947) in Haiti; Carr and Hill (1942) for Cuba; and Boyd & Aris (1929) in Jamaica.

DISCUSSION

In their note on *A. albimanus* from Trinidad and Grenada, Howard, Dyar, and Knab (1935) postulated that *Anopheles* occurring ". . . in the other islands

(of the Antilles) will be found to be *tarsimaculatus*," but in view of the subsequent observations recorded above it is clear that although *A. tarsimaculatus* (*aquasalis*) does occur in those islands, *albimanus* is absent from many, including Trinidad and Grenada.

For St. Vincent, the earlier listing of this species by Senevet (1936) is now recognized as an instance of confusion with *tarsimaculatus* (*aquasalis*), and although a later unpublished report of *albimanus* was made by Seagar (1939) on adult specimens from this island, examination of a large and representative sample of *Anopheles* larvae during the favorable dry season of 1944 (Charles 1952) failed to confirm this. No control measures had been instituted in the interval between the two observations.

The Barbados record has not been subject to review either by subsequent collections or re-examination of original specimens, which were not preserved. From Seagar's larval descriptions no definite conclusions can be drawn, since the dorsal submedian prothoracic hairs were not described. His adult identifications were based primarily on ". . . white markings on the palpi (which were) long and dark—last segment *and base* of penultimate one white—. . ." But Komp (1942) described the palpi of *A. albimanus* as having ". . . terminal segment white, penultimate segment black . . . *antepenultimate segment black with a narrow white band at tip* . . ."; and in *A. aquasalis* they have ". . . terminal segment white; penultimate segment narrowly black at base and tip, middle with pure white scales. . . ."

If it be conceded that Seagar's "base of penultimate segment" referred to Komp's "tip (apex) of antepenultimate segment," then the Barbados species was probably *albimanus*. But since Seagar (1939) apparently confused *aquasalis* adults from St. Vincent for *albimanus*, the Barbados identification must remain in doubt.[3]

In the light of more recent observations the 1936 reference to *A. albimanus* in St. Lucia and Dominica must be considered equally erroneous to that for St. Vincent, for, it must be emphasized that the author (Senevet) did not himself make any collections in those islands. Indeed, these references were based on Theobald's (1910) listing of *Cellia albimana* in these three islands, and whereas in 1936 this was interpreted as being synonymous with *Anopheles albimanus*, it was later realized that Theobald was actually referring to *A. tarsimaculatus* Goeldi which is now recognized as *A. aquasalis* Curry.

Since Nicholls' (1912) description of *albimanus* adults would apply equally to *aquasalis*, and his use of larval color markings was admittedly unreliable, this record also must now be corrected.

In the publication on Guadeloupe, Senevet (1936) suggested the evidence on which the presence of *A. albimanus* in the Lesser Antilles should be primarily based, viz. the characteristics of the male hypopigium, and inner (No. 1) dorsal submedian prothoracic larval hair. Today Senior-White (1952) makes use of

[3] After submission of this manuscript we first became aware of the existence of undoubted specimens of *A. albimanus* collected on Barbados in 1928, as recorded by Dr. L. E. Rozeboom in the ensuing paper in this number of American Journal of Tropical Medicine and Hygiene.

the maxillary index as a reliable guide in differentiating between adult *albimanus* and *aquasalis*—even on a single specimen—but the reliability of larval identification is also acknowledged.

During the survey of Dominica and St. Vincent (Charles 1952) only a small number of male terminalia were dissected, all of which conformed with the characteristics of *aquasalis*. The majority of the identifications, however, both here and in the islands further north as far as St. Kitts, were based on larval examinations in which the presence of a stout heavily branched inner sub-median prothoracic dorsal hair was usually adequate to determine the occurrence of *A. albimanus*. For female adults, especially in those islands such as Montserrat, Antigua and Nevis where both *aquasalis* and *albimanus* occurred, reliance was placed on the presence of apical and basal black rings and all-white middle on the penultimate palpal segment to differentiate *aquasalis* from *albimanus*.

CONCLUSIONS

From the foregoing it is concluded that in the Caribbean island chain *A. albimanus* does not extend southwards beyond Longitude 15°53′ N. in the French island of Marie Galante, immediately southeast of Guadeloupe in the Lesser Antilles. Its earlier report in Trinidad, Grenada and St. Lucia has long been disproved and the data here presented are submitted as similar proof of its absence from St. Vincent, St. Lucia and Dominica. Its citing in these islands resulted from a confusion of synonymous nomenclature for Theobald's *Cellia albimana*. Its probable importation to Barbados during 1928 remains uncertain, but it is not now present in that island.[3]

The interesting and sudden cessation of *A. albimanus* at Marie Galante, 16 miles north of Dominica was commented upon by Senior-White (1952) who pointed out that whereas Marie Galante belongs to the nonvolcanic island arc up to Guadeloupe, Dominica is part of the volcanic arc. The distribution of this mosquito species in this area is therefore probably related to the geological history of the islands. On the other hand, its failure to bridge the narrow gap between Guadeloupe and Dominica—even in recent times when an appreciable flow of traffic by small craft exists between these two islands—remains unexplained, and is somewhat comparable to the absence of ten species from Tobago which occur in Trinidad (Senior-White 1950).

In view of the data here presented, Senior-White's (1950) table of Distribution of Anopheles around the Caribbean Sea should be amended to show the presence of only the two species *A. aquasalis* and *A. argyritarsis* in St. Vincent and Dominica.

SUMMARY

1. The presence of *A. albimanus* in the island units of the Caribbean is discussed.

2. Field observations have now established its absence from the Netherlands Leeward and Windward Islands, Trinidad and Tobago, Grenada, St. Vincent, Barbados, St. Lucia, Martinique, Dominica and St. Kitts. The southernmost

point of its distribution in the island chain is established in Marie Galante at latitude 15°53′ N.

3. In the Caribbean Islands *A. albimanus* is limited to Guadeloupe (and Marie Galante), Montserrat, Antigua, Nevis, Virgin Islands (St. Croix, St. Thomas and Tortola) and the Greater Antilles.

REFERENCES

BEATTIE, MARY V. F. 1932, The physico-chemical factors of water in relation to mosquito breeding in Trinidad, *Bull. Ent. Res.* **23:** 477–500.

CAUBET, P., ABBONENC, E., BOISNEUF, R. AND SYDNEY, L. 1948, Les moustiques du genre anophèle à la martinique, *Arch. Inst. Pasteur de la Martinique* **1:** 9–11.

CHARLES, L. J. 1952, Malaria in the Leeward and Windward Islands, British West Indies. *Am. J. Trop. Med. & Hyg.* **1:** 941–961.

DOWNS, W. G., GILLETTE, H. S. P. AND SHANNON, R. C. 1943, A malaria survey of Trinidad & Tobago, British West Indies, *J. Nat. Mal. Soc.* **2:** Suppl., 5–44.

EARLE, W. C. 1930, Malaria in Puerto Rico, *Am. J. Trop. Med.* **10:** 207–230.

—— 1936, The relative importance of *Anopheles tarsimaculatus*, *Anopheles argyritarsis* and *Anopheles pseudopunctipennis* as vectors of malaria in the Windward Group of the West Indies, *Am. J. Trop. Med.* **16:** 459–469.

EDWARDS, F. W. AND BOX, H. E. 1940, Notes on mosquitoes, *Ann. & Mag. Natural Hiitory*, Series 11, **5:** 314–322.

FLOCH, H. AND ABONNENC, E. 1945, Les moustiques de la Guadeloupe, *Arch. Inst. Pasteur Guyane*, Publ. No. 108, 1–16.

GABALDON, A. 1949, Malaria incidence in the West Indies and South America, Contributed in "*Malariology*", M. F. Boyd *et. al.* Vol. 2, pg. 766., W. B. Saunders, Phil. & London.

HOFFMAN, W. A. 1930, From San Juan to Aruba, *Puerto Rico J. Publ. Hlth.* **5:** 357–369.

HOWARD, L., DYAR, H. AND KNAB, F. 1915, *The Mosquitoes of North and Central America and West Indies*, Vol. 3, pg. 978. Carnegie Inst. of Washington.

JULIEN, G. FRANÇOIS. 1930, De la persistence du paludisme à la Guadeloupe, Medical Thesis, Paris (Unpublished).

KOMP, W. H. W. 1937, *South. Med. J.* **30:** 946 (Discussion of a paper).

—— 1942, The anopheline mosquitoes of the Caribbean Region, *Nat. Inst. Hlth. Bull.* No. 179, pp. 67 & 73, Govt. Printing Office, Wash.

NICHOLLS, L. 1912, Some observations on the bionomics and breeding places of Anopheles in Saint Lucia, British West Indies, *Bull. Ent. Res.* **3:** 251–267.

ROOT, F. M. AND ANDREWS, J. 1938, Malaria and anopheline survey of Grenada, British West Indies, *Am. J. Hyg.* **27:** 549–579.

ROZEBOOM, L. E. 1941, *A Symposium on Human Malaria*, p. 99, Publ. No. 15, Am. Ass. Adv. Sci., Wash. D. C.

SEAGAR, E. A. 1928, Malaria in Barbados, *Trop. Agriculture*, Trinidad, **5:** 48–50.

—— 1939, Unpublished.

SENEVET, G. 1936, Les moustiques de la Martinique, *Arch. Inst. Pasteur d'Algerie* **14:** 123–134.

—— 1938, Les moustiques de la Guadeloupe, *Arch. Inst. Pasteur d'Algerie* **16:** 176–190.

SENIOR-WHITE, R. A. 1950, The distribution of the Culicid tribe Anophelini around the Caribbean Sea, *Caribbean Med. J.* **12:** 3–7.

—— 1952, Personal communication.

THEOBALD, F. V. 1910, *A Monograph of the Culicidae of the World*. Vol. V. pg. 69. London, Brit. Mus. (Nat. Hist.).

VAN DER KUYP, E. 1949, Annotated list of mosquitoes of the Netherlands Antilles, including French St. Martin, *Documenta Neer. et Indonesica de Morb. trop.* **1:** 69–70.

A NOTE ON THE PRESENCE OF *ANOPHELES ALBIMANUS* IN BARBADOS

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In the preceding paper Charles and Senevet raise a question concerning the accuracy of the identification of *Anopheles albimanus* which Seagar had reported from Barbados. Among the material in the collection of the Department of Parasitology, the Johns Hopkins University, there are four pinned adults (one male and three females) bearing the following label: "A. albimanus—Barbados—1928. Received Apr. 24." There is also a slide mount of the terminalia of two males, and another slide on which is mounted some first, second, and two third stage larvae. These slides are labelled "Anopheles albimanus. Barbados, 1928" in the late Dr. F. M. Root's handwriting. Presumably all this material had been sent to Root for confirmation of identification, so that these specimens probably represent some of the original material taken on Barbados by Seagar.

Two of the females are in good condition and I would identify them without hesitation as being *A. albimanus*. The fourth hind tarsal segment is a little more than half dark; the fourth palpal segment is dark and not marked with white scales such as would be found on *A. aquasalis*; the second white humeral spot is larger than the black spot just basal to it, and the tip of vein 2.2 is white. The abdomen of the male is missing; one would imagine that one of the two mounted terminalia belongs to this specimen. Both of these terminalia show the typical fused ventral claspette lobe of *albimanus*, with a median sulcus and bladder-like lateral expansions. The two third-stage larvae can also be determined as *A. albimanus*, as the inner submedian prothoracic hair has a short thick main stem and a number of long slender branches. This can not be confused with the short palmate-like prothoracic hair No. 1 of *A. aquasalis*.

These specimens prove that *A. albimanus* was present on Barbados at the time that Seagar made his survey. They do not alter the conclusion reached by Charles and Senevet that this species now is absent from the island. It is of considerable interest that this hardy mosquito had become established on an island close to its natural range, which should be favorable to its survival; yet it died out. If the factors responsible for its extermination could be determined, we might possess knowledge that would be of value in planning control or eradication measures elsewhere.

A PRELIMINARY ANALYSIS OF GARBAGE AS FOOD FOR THE NORWAY RAT[1]

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Investigators in the field of population mechanics have long recognized the need for a quantitative approach to the food factor, for it has already been established that food *per se* can be and sometimes is a limiting factor in population growth. Allee *et al.* (1949) explicitly state: "The principle that animal populations tend to be limited by their food involves the corollary that populations tend to expand in the presence of available food." Malthus (1798) observed that many human populations are limited by the total amount of available food, and based his famous "Essays on Population" on this premise. Many examples are available in the literature today bearing on food as a limiting factor. However, in spite of the general agreement on the importance of this factor in the ecology of animal populations, very little has been done to make quantitative observations on this point.

During the summer of 1950 an opportunity arose to conduct some preliminary investigations on the relationship between a measurable food supply (garbage) and a measureable population of Norway rats (*Rattus norvegicus*), which was followed by some controlled experimental investigations into the utilization of various types of foods by the rats. This latter work was made possible by the cooperation of Morgan State College, Baltimore, Maryland, in making available to the writers a deserted storage barn on the college grounds, and also by donating a large variety of leftover foods and garbage without which the latter part of this investigation would have been impossible.

The object of these investigations was to determine both the quantity and quality of food required by an individual rat living in its normal environment. It must be emphasized at this point that this was merely a preliminary investigation and that more detailed and more carefully controlled series of measurements are necessary before we can approach a prediction of numbers of rats from pounds of refuse.

The writers wish to express their sincere gratitude and appreciation to the staff of the cafeteria at Morgan State College and also to Dr. Irwin Bross for his suggestions and guidance with the statistical work involved. Dr. David E. Davis offered many helpful suggestions during the course of the investigation.

QUANTITY OF FOOD AVAILABLE

In urban communities where natural rat-foods have been restricted by the construction of buildings and the paving of streets, the rat has become largely

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dependent upon domestic refuse as a source of food. Environmental surveys of rat-infested city blocks indicated in a large majority of cases that a positive correlation existed between the availability of refuse as food for rats and the degree of infestation (Orgain and Schein, 1953). In order to get a clearer picture of the quantity of food available to the rat, it was necessary to determine both the total quantity of refuse in a typical block and the proportion of the refuse which was edible.

A representative residential city block in Baltimore was selected for these measurements. The rat population of this block had been measured at bimonthly intervals from March, 1949, until the beginning of this study in March, 1950, and was essentially stationary at about 105 rats. The population measurements were conducted according to the methods developed by Emlen, Stokes and Davis

TABLE 1

Quantity of refuse and the per cent garbage produced in a city block

MONTH	NUMBER OF COLLECTIONS	TOTAL WEIGHT OF REFUSE	WEIGHT OF SAMPLE QUARTERS	PER CENT EDIBLE GARBAGE IN SAMPLE QUARTERS
		lbs.	*lbs.*	
June	8	12,829	347	26.5
July	6*	9,946	156	33.9
August	8	10,054	360	34.2
September	8	11,412	778	28.9
October†	3	3,715	128	35.2
Totals	33	47,956	1,769	31.7 average

* Two holidays on which collections were not made.

† 1½ weeks of October.

(1949). The total refuse of the block was measured by actually weighing all the bags, boxes, and cans of refuse immediately prior to the semiweekly collections by city crews, being careful to subtract the weights of the empty cans and boxes that were reused. To determine the per cent edible, 6 to 8 containers filled with refuse were randomly selected throughout the block just preceding collections, their contents mixed thoroughly in one large heap and one-fourth (by volume) of the mixture randomly selected for detailed separation into edible and inedible components. Both portions of the sample were then weighed and the resultant per cent garbage of the sample was considered as representative of the block for that collection period. Sampling in this manner was carried on for 18 weeks and the results are shown in Table 1.

The authors estimate that in this typical city block, at least ⅓ of the total refuse was always available to the rats (i.e., not in ratproof containers). Since the rat population was about 105 rats at the time, simple arithmetical manipulations of the figures in Table 1 indicate that each rat was supplied with about ⅓ lb. (150 gm.) of edible garbage per day. If this ration was barely adequate to the rat's needs, then certainly food quantity would probably be governing the

population level of the block. If the ration was excessive, then some other environmental factor, perhaps food *distribution*, might be governing the population level. Therefore the problem was resolved into determining just what "150 grams of garbage" means in terms of rat nutrition and palatability.

QUALITY OF AVAILABLE FOOD

It should be emphasized at the outset that the work reported on the following pages is of a preliminary nature, and that a great deal of more closely controlled experimentation is necessary before widely applicable conclusions can be reached. Essentially, this was a search for a method of attacking the problem and should not be misinterpreted as the attack itself.

Basically, the experimental design governing this investigation is as follows: the foods commonly found in domestic refuse were individually fed to groups of caged rats for a set period of time (3 days). The data obtained for each food item consisted of the total quantity of test food eaten, the gain (or loss) in weight of the test rats, and a relative measure of the preference of the rats for the test food over a standard diet. The foods were ranked according to their caloric content, and an attempt was made to determine roughly the amount of food necessary for the rat to survive. The details of the procedures and results obtained are presented below.

METHODS

The rats. The rats used in these experiments were trapped unharmed in various residential blocks in the city of Baltimore, Maryland, and included representatives of many different colonies in the wild. Five to eight rats (depending upon their combined weights), randomly distributed with respect to age and sex, were placed in a cage. Each rat was marked individually by clipping its toes and weighed at predetermined intervals. The rats were grouped in order to try to eliminate individual variations in food intake. It is assumed that the effects of individual variations in consumption due to position in the social hierarchy were overcome by calculating all the analyses on a group basis. (This assumption may be quite invalid and will require further study; for the present it must be by-passed, although not ignored.) Six to seven cages were used, with a total weight of from 1000–2000 grams of rats per cage.

The cages consisted of two 3 x 3 foot compartments, the door between the compartments being always open; water was available *ad lib.* The barn in which the cages of rats were kept was fairly well isolated from other inhabited structures and, except for an occasional inquisitive youngster, the caged rats were not unnecessarily bothered. In all, conditions indicated that the rats were subjected to a minimum of disturbances which might have affected their overall food intake.

The temperature of the barn was not measured, although it was always somewhat cooler inside than the surrounding outdoor area. The rats were kept in the barn from the end of August to the beginning of November, 1950, and at no time did the temperature approach freezing. Humidity, although not measured, was

fairly high. After each rain, water ran in natural rivulets into the barn through gaps under the door and consequently the interior of the barn was almost always wet and muddy during the period of the investigation. The rats were subjected only to natural illumination through the open barn windows or doors, but at no time could direct sunlight fall on any cage. In all, temperature, humidity and light were assumed not to play any critical role affecting the feeding behavior of the rats.

Animals that were removed for reasons of sickness or death were not replaced by others unless the gross weight of the group of rats in that cage fell below 1000 gm. In the event that replacing was necessary, no feeding experiments were run on that particular group for at least a week to give the introduced rats time to adjust to their new environment. It was hoped by these means to eliminate or minimize the aberrant results which might be produced by a group undergoing violent upheaval or adjustment of the social order.

The foods. The foods that were used in the experiments were designed to represent those items normally encountered in domestic refuse. Since large quantities of single foods were necessary for each test run, most of the items were collected from the discards of rather large institutions such as local supermarkets, the Baltimore Produce Freight Terminal and, in the case of prepared foods, from the cafeteria leftovers at nearby Morgan State College. Certain foods such as potato peels and melon rinds were available in sufficient quantity directly from the test blocks.

An analysis of the various foods was prepared for the writers by Miss Janette Carlson, chief dietitian of the Johns Hopkins Hospital. This analysis, taken largely from Bowes and Church (1946) and Bridges and Mattice (1950) listed many properties of each food item. In view of the preliminary nature of this investigation, only the protein, fat and carbohydrate content of the foods were used, and for convenience of analysis these three properties were combined according to their energy ratios. Thus each food was listed in terms of its caloric content per hundred grams of food. This value, hereinafter referred to as *cal*, offered a convenient and reasonably precise method whereby one food could be compared with others and at the same time with the various measurements derived from the rats.

It was also desirable to compare the rats' reactions to a particular food with their reactions to a "standard" food. A laboratory diet, "Fox Checkers" (manufactured by the Ralston Purina Company) was used as the standard because of its availability, completeness in diet and fair consistency in composition. This standard is represented as having 315 calories per 100 gm. Fox Checkers (or *cal* = 315), which was somewhat higher than most of the experimental foods used.

Feeding the rats. The individual feeding experiments were conducted on a 7-day schedule, with three different measurements obtained each week on each cage of rats. Generally, every Tuesday, Wednesday and Thursday were set aside for the measurement of the consumption of a particular food, while Friday, Saturday and Sunday were used to measure the preference of the rats for a different food as compared with the standard. Monday was used as a day of recovery and

standardization, during which only the standard diet was offered to all cages. A measure of how well or how poorly the rats fared on a particular diet was obtained from their weight records before and after the consumption part of the test.

As an example, on September 19, 20 and 21, 1950, Cage II was offered no other food but cantaloupe, while Cage III was offered only macaroni-and-cheese. On September 22, 23, and 24, 1950, Cage II was offered onions together with the standard Fox Checkers, while Cage III was offered raw cauliflower and standard. (In only a minimum of cases was the same group of rats used for both a consumption and preference test on the same food.) On the last day of this particular cycle, September 25, 1950, all cages were offered only Fox Checkers, in an effort to standardize them as much as possible for the next series of tests.

Actually there is a recovery period of four days from the end of one monofood diet to the beginning of another, for during the three days of preference testing there was enough of the standard available to compensate for any loss in weight incurred as a result of a poor diet the previous three days. An examination of the total weights of the grouped rats at the beginning of each consumption test shows that, in general, each suceeding week involves a somewhat heavier group of rats, indicating not only complete recovery from a poor diet but in addition a gain in weight that would be expected with normally fed rats. Hence, although the problem of "normal recovery" was not dealt with as fully as it probably warranted, it was assumed that the cages of rats were fairly well standardized at the start of each experimental series.

Replication of measurements was not possible at this time due to the limited quantities of food that were available. However, the standard diet was fed to three groups simultaneously in order to get some indication of the inherent variation of the indices. The fact that there is but a bare minimum of replication makes a complete statistical analysis of the results impossible; this deficiency should be obviated by more detailed and refined tests.

Measurements and definitions. 1. *Weight, w:* How well or how poorly the rats fared on any food item was determined by calculating the group's gain or loss in weight when fed that particular food item alone. (The same set of scales, precise to about 4 gm., was used to weigh both the rats and the foods.) Thus, the weight index, w, for each food is hereby defined as:

$w = \frac{w_f}{w_0}(100)$, where w = weight index

w_f = combined weight of the group of rats at the end of the 3 day consumption period.

w_0 = combined weight of the group of rats at the beginning of the 3 day consumption period.

For example, on September 19, 20 and 21, 1950, Cage II was offered cantaloupe alone. The initial weight of the group, w_0, was 1358 gm. and the final weight weight, w_t, was 1250 gm. Therefore, the w index for cantaloupe, according to the above definition would be 92.1.

2. *Consumption, c:* The foods were offered to the rats in weighed portions,

and the amount of each food consumed was recorded daily. In all cases there was a surplus of food, and in cases of perishable foods every effort was made to insure the rats a fresh supply daily. A consumption index, c, was calculated for each food after feeding that item alone for 3 days. Thus,

$$c = \frac{c_1 + c_2 + c_3}{3g} \text{ where } c = \text{consumption index}$$

c_1 = total grams of food item consumed by the group on the first day.
c_2 = total grams of food item consumed by the group on the second day.
c_3 = total grams of food item consumed by the group on the third day.
g = average weight of the group of rats for the 3 day period, (in hundreds of grams).

The amount of error introduced by evaporation of water from the foods was believed to be greatly reduced because of the high humidity within the barn. For this reason, no attempt was made to try to determine the magnitude of this error. Of greater importance was the possibility of dry foods, such as cornmeal, picking up moisture in the cages. This was partially solved by keeping the stock supplies of dry foods exposed in the barn for a few days before feeding.

To continue the above example (cantaloupe), the arithmetic average weight of the rats for the 3 day period would be (1358 + 1250/2) or 1304, and therefore $g = 13.04$. The consumption on the first day was 635 gm.; on the second day, 890 gm.; on the third day, 810 gm. Therefore, by applying the above formula, we get $c = (635 + 890 + 810/(3)\ (13.04)) = 59.69$.

3. *Preference, p:* As indicated above, the second 3 days of the 7 day schedule was set aside for preference tests. An index of preference, p, was calculated for each food, p representing the ratio of grams of test food consumed per hundred grams of standard, when both are offered simultaneously and both in excess for 3 days. For example, on September 22, 23 and 24, 1950, Cage III was offered raw cauliflower and standard diet, both in surplus quantities. In the three day period, 290 gm. of the raw cauliflower and 570 gm. of the standard diet were consumed. The resulting preference value p, equals 290/5.70, or $p = 50.9$.

Armed with an evaluation of each food item in terms of w, c, p and *cal*, it is now possible to proceed to a consideration and analysis of the results.

RESULTS AND DISCUSSION

Table 2 shows the foods used, their caloric content as obtained from references, and the calculated w, c, and p value of each food. In addition, there was a group of foods for which an energy analysis was not readily available; these foods are listed in Table 3, and since a *cal* index was not obtainable they will not be considered further.

Estimate of variation of measurements. In order to get an indication of the amount of variation between cages of rats, three groups were simultaneously fed the standard diet, using the same routine as a consumption test; the results are shown at the end of Table 2.

TABLE 2

The cal, c, w *and* p *values of the foods used*

FOOD ITEM	*cal*	*c*	*w*	CAGE NO.[a]	*p*	CAGE NO.[b]
Raw cucumber	15	68.4	84.0	I	154.7	II
Raw celery	16	39.5	84.5	I	30.2	IV
Lettuce	18	23.0	90.6	III	95.2	II
Radish (with tops)	21	31.4	84.6	V	59.6	IV
Raw cauliflower	22	39.8	90.0	V	50.9	III
Cantaloupe	22	59.7	92.1	II	283.5	IV
Raw tomatoes	23	53.4	88.7	IV	132.1	II
Cooked spinach	25	49.4	89.1	VI	61.3	I
Raw eggplant	28	36.3	85.6	VI[c]	124.5	VI[c]
Raw cabbage	29	27.5	83.1	VI	58.2	V
Cooked cabbage	29	74.3	77.4	I	78.3	II
Green peppers	29	59.5	85.6	IV	54.5	III
Cooked carrots	30	92.8	91.0	II	204.2	IV
Cooked cauliflower	31	74.9	88.2	II	36.2	VI
Raw stringbeans	42	22.1	89.4	V	—[d]	—
Cooked stringbeans	42	37.9	83.3	VI	88.9	IV
Grapefruit	44	39.1	87.3	VI	50.0	III
Raw carrots	45	38.8	85.8	IV & V	54.1	I
Raw beets	46	43.9	89.0	I	20.9	III
Onions	49	8.4	84.0	III	24.0	II
Oranges	50	27.1	87.0	III	95.3	IV
Peaches	51	—[d]	—[d]	—	23.9	VII
Plums	56	—[d]	—[d]	—	70.2	V
Apples	64	35.3	89.0	III	82.6	VI
Grapes	74	40.4	88.4	V	119.4	I
Cooked corn kernels	77	33.8	98.6	III	1027.6	V
Raw potato peels	85	23.3	89.2	IV	50.9	VI
Raw potatoes	85	29.0	97.3	IV	84.1	VI
Cooked potatoes	85	47.6	102.0	I	720.0	VII
Bananas	99	53.9	91.8	IV	188.6	I
Raw corn on cob	108	47.8	101.6	IV	315.0	II
Cooked oatmeal	119	45.4	101.4	VI	680.5	V
Raw sweet potatoes	125	31.9	91.8	VII	266.0	VI
Cooked sweet potatoes	125	14.4	103.3	V[e]	492.3	V[e]
Corned beef hash	143	15.2	96.2	I	252.9	VI
Macaroni-and-cheese	154	24.1	104.7	III	1055.0	I
Chili con carne	198	17.3	97.8	I	89.3	II
Boiled chicken	200	30.8	105.0	IV	—[d]	—
Fried chicken	224	18.1	108.6	VI	228.6	V
Scrambled eggs	253	17.3	100.3	VI	4950.0	IV
Raw beef with bones	255	15.2	—[d]	II	342.9	IV
White bread	261	12.6	96.3	V	451.6	I
Raw beef, no bones	277	21.0	101.3	II	244.8	VI
Cooked ham	296	6.9	91.4	III	95.2	V
Cooked beef	332	10.7	101.6	VI	—[d]	—
Ground cornmeal	351	10.0	98.0	II	327.8	III
Cooked pork with bones	392	11.3	102.6	I	181.5	IV
Standard lab diet	315	10.4	107.1	II		
Standard lab diet	315	10.2	103.6	III		
Standard lab diet	315	10.4	96.3	I		

[a] Group used to measure *w* and *c*.

[b] Group used to measure *p*.

[c] 15 days elapsed between the consumption and preference test.

[d] Not measured.

[e] 47 days elapsed between consumption and preference test.

It is at once apparent that although the *c* index is relatively constant for each of the 3 groups, the *w* index shows considerable variation. Hence, *w* indices were

TABLE 3

Foods not used in the analysis; their calculated w, c *and* p *values*

FOOD ITEM	c	w	CAGE NO.[a]	p	CAGE NO.[b]
Veal hearts, raw	19.6	95.1	III	—[c]	—
Raw mixed vegetables[d]	31.8	87.3	II	97.0	V
Horse feed[e]	6.4	96.9	V	237.2	IV
Watermelon rind[f]	63.4	86.0	II	—[c]	—
Honeydew melon rind[f]	48.8	90.5	V	465.3	I
Lima bean hulls	10.2[g]	83.4[g]	VI	—[c]	—
Cooked corn cobs[h]	31.8	84.9	IV	153.5	III

[a] Group used to measure *w* and *c*.
[b] Group used to measure *p*.
[c] Not measured.
[d] A commercial preparation sold primarily for soup stock.
[e] Consisting mainly of corn, oats, and molasses.
[f] A substantial amount of melon was included with rind.
[g] This experiment terminated after 2 days because of the severe starvational effect on the rats.
[h] Prepared to simulate garbage can conditions.

TABLE 4

Variation in w *on a normal (Fox Checkers) diet*

RAT NO.	SEX	CAGE	w_0	w_f	w FACTOR
13-1; 0-1	M	I	430	392	91.1
13-1; 0-2	M	I	350	338	96.5
12-2; 0-4	M	I	364	362	99.4
12-1; 4-0	F	I	174	156	89.7
12-0; 0-3	F	I	388	380	97.9
12-0; 0-1	F	I	160	168	105.0
12-1; 0-2	M	II	438	464	105.9
12-0; 4-0	M	II	200	222	110.9
12-1; 3-0	F	II	170	184	108.2
12-1; 2-0	F	II	164	170	103.8
12-1; 0-1	M	II	400	430	107.3
12-3; 4-0	M	III	470	482	102.6
12-3; 1-0	F	III	180	182	100.5
12-3; 3-0	F	III	232	250	107.7
12-3; 0-1	F	III	390	402	103.1
12-3; 2-0	F	III	384	400	104.1

Mean w = 102.1. Standard deviation = 5.91. Standard error of the mean = 1.50.

calculated for each of the 16 rats involved, in an attempt to establish the basis for this somewhat unexpected variation; these data are shown in Table 4. An examination of this table indicates that some group phenomenon had biased the

results of Cage I rats to such an extent that 5 of the 6 rats gave w's not comparable to the other 10 rats in the experiment. It is interesting to note, though, that the c index of Cage I did not differ materially from the other 2 groups.

Because of the preliminary nature of these experiments, it was not deemed advisable to pursue the question of sources of variation any further. It is enough to recognize at this time that before any experiment of this nature can be considered successfully completed, a thorough consideration of variability would be necessary.

The relationships between the indices. Figure 1 shows the relationship between w and *cal*, plotted on semilog paper; the two lines are the calculated regression lines. The coefficient of correlation, r, for these indices is $+0.76$ ($P < 0.01$).

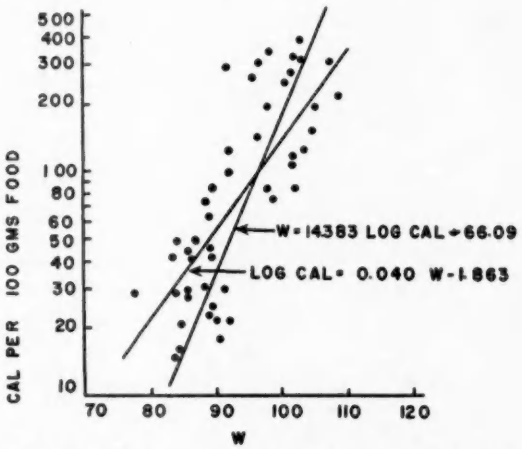

FIG. 1. The relationship between w and *cal*, showing the calculated regression lines

Assuming that a group will not survive unless it maintains or gains weight ($w \geq 100$), then from the regression formulae shown in Figure 1, about 137 calories per 100 gm. food would be the minimum energy requirement for group maintenance (Log $cal = 0.040w - 1.863$; $w = 100$). Now if it were possible to get an estimation of the average caloric content of the block's refuse, we would be able to estimate the amount of food that would provide 137 calories. However, such an estimation is not available at this time, and so we must resort to a somewhat roundabout method of calculating the rat's needs: utilizing the relationship between *cal* and c (Figure 2).

Although the points are widely dispersed (Figure 2), they are somewhat suggestive of a hyperbolic function of the general formula $cal \times c = k$, where k is some real, finite constant. This formula would suggest that rats strive for a definite amount of food *value* (per 100 gm. body weight) daily. In other words, a rat would seek his *nutritional* fill rather than his *volume-capacity* fill. With some foods, such as cooked cabbage or cooked carrots (see Table 2), the rat would be more

likely to reach his capacity fill before his nutritional needs are met. On the other hand, some of the other foods, such as ground cornmeal or standard laboratory diet, may well satisfy the rat long before his volume-capacity is reached.

Assuming that $cal \times c = k$ is a valid interpretation, it is possible to calculate k in terms of calories consumed per 100 gm. body weight per day. Using the food items listed in Table 2, $k = 25.87$ (confidence limits, $P < 0.05$: $k = 21.27$; $k = 30.47$). Combining k with the calculated minimum of 1.37 calories per gram of food necessary for survival, we find that the rats would require 18.88 gm. of moderately high caloric food per 100 gm. body weight per day.

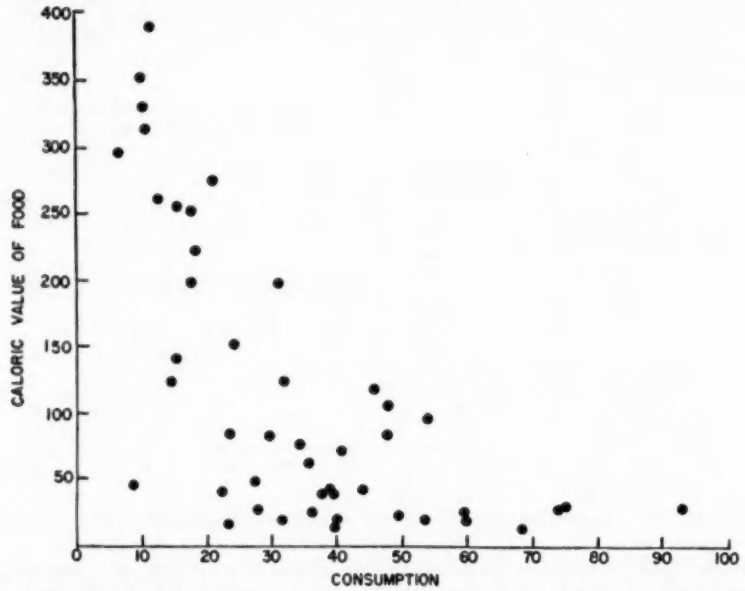

FIG. 2. The relationship between *cal* and *c*, indicating a possible hyperbolic interpretation

To speculate even a bit further, it was estimated from field observations (see above: Quantity of food available) that there was about 150 gm. of edible garbage available per rat per day. It is also reasonable to assert (from personal experience) that a goodly portion of this food, probably about half, falls below the required minimum caloric content of 137 calories per 100 gm. food. Since the average (250 gm.) rat would require about 47 grams of food daily, it is apparent that the actual supply of food is about 1½ times the basic requirement. In a previous report (Orgain and Schein, 1953) it was pointed out that the garbage supply of this block was reduced an estimated 50 per cent before there were any visible effects on the rat population. Allowing for variations from many possible sources, that estimate seems to agree fairly well with these calculations. It has been found (Chitty, 1942; Emlen, Stokes and Davis, 1949, and others) that rats

in nature will eat about 25 to 30 grams of wheat or corn, which agrees reasonably well with the 47 gm. estimate calculated above. Note from Table 2 that a 250 gram rat would eat 31 grams of white bread per day. Therefore, if food was limiting population expansion in this block, it was apparently doing so through the mechanism of limited distribution, rather than limited quantity. (See Orgain

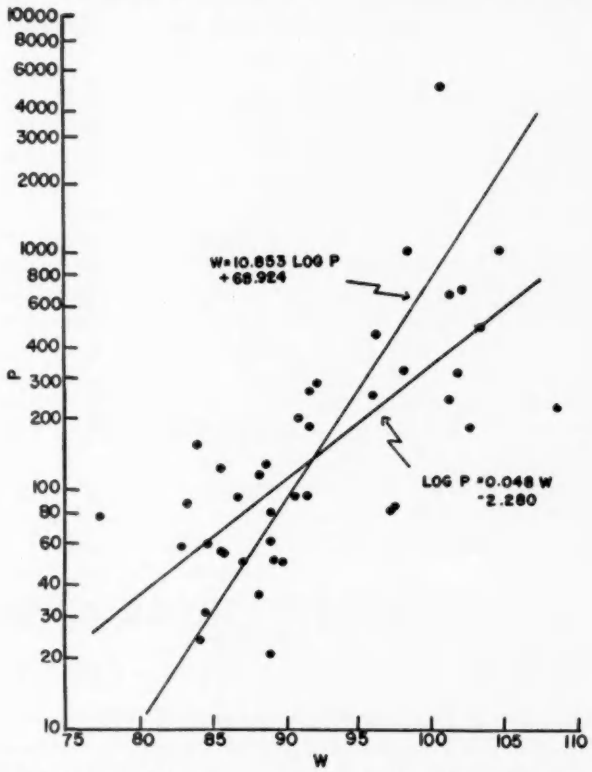

FIG. 3. The relationship between w and p, showing the calculated regression lines

and Schein, 1953, for a further discussion of food distribution affecting rat populations.) Admittedly these speculations are plagued with many assumptions, some of which may be open to serious question. However, as mentioned at the outset, the conclusions arrived at here can be useful only as a guide to further investigations.

Rat food preferences. During the course of the above described experiments, a comparison of the test food with a standard diet was readily available. This has been termed the preference ratio, p. The relationship between p and w is shown in Figure 3; the correlation between these indices is significant ($r = +0.72$, $P < 0.01$). Broadly interpreted, this would indicate that the rats generally pre-

fer foods that they will gain weight on, e.g., that are ultimately good for them, and would tend to avoid foods that are not useful to them.

It is interesting to speculate as to why scrambled eggs should have a p so far out of proportion to its w value (Table 2). It has been mentioned above under Methods that at no time during the course of the investigation could direct sunlight reach any of the caged rats and it is entirely possible that the rats were becoming Vitamin D deficient. Scrambled eggs was the only food offered that

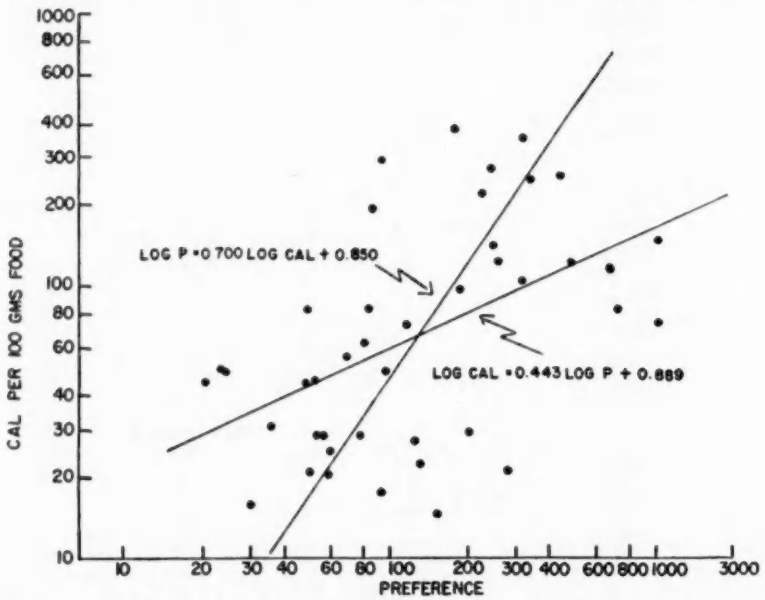

FIG. 4. The relationship between *cal* and *p*, showing the calculated regression lines

contained more than trace amounts of this vitamin. Whether there is any correlation between the high p for scrambled eggs and the possible avitaminosis of the rats requires much further study.

There is no readily apparent explanation for the relatively low p values of fried chicken, chili con carne and cooked ham, except that it is possible that the rats were somewhat repelled by the excessive seasoning of these foods.

Since there exists an apparently linear relationship between w and log *cal* (Fig. 1) and also between w and log p (Fig. 3), we would expect to find a linear relationship between log *cal* and log p. When these two factors are plotted against each other the points are subject to much scatter, as is seen in Figure 4. The calculated regression lines are again shown in the figure; $r = 0.557$ ($P < 0.05$). Apparently the methods used in this investigation were not precise enough to show this relationship clearly, especially since sources of large variation, such

as food seasoning, were beyond the control of the authors. On the other hand, the fact that there is such a large degree of scatter in both Figures 3 and 4 may well emphasize the individual rat's "tastes"; the problem at this point is left to the competence of the psychologist. Because of the preliminary nature of this investigation, and because no estimate of the variation in measurement is possible, it was not deemed advisable to test this relationship further by the method of multiple regressions.

SUMMARY

The total quantity of domestic refuse from a typical residential city block was measured for a period of 18 weeks during the summer of 1950. Samples of the refuse were selected at random for analysis and it was found that approximately 30 per cent of the refuse (by weight) was edible. Assuming that about ⅓ of this ration is always available to the rats, it is estimated that each rat was supplied with about 150 gm. of edible material per day.

For a more detailed analysis of the particular food items, 50 foods were selected on the basis of their ready availability to the rats in domestic garbage and were fed to groups of caged rats. The caloric content per 100 gm. of test food (*cal*) was obtained from the literature. In addition, three indices were calculated for each food from the results of 3-day feeding trials. The *w* index represented the percentage, at the end of a 3-day diet, of the rat's initial body weight. The *c* index represented the average daily consumption, per 100 gm. rat body weight, of a particular food, when no other food was available for a 3-day period. The *p* index represented the number of grams of test food eaten per hundred gm. of a standard food, both being offered simultaneously and to excess for 3 days. The standard food used was "Fox Checkers," a complete-diet laboratory food manufactured by the Ralston Purina Company.

The problem of replication of measurements could not be dealt with as fully as might be desired. However, in order to get some indication of the magnitude of variation inherent in the indices, the standard diet was fed to 3 groups simultaneously. The results showed a considerable amount of variation in *w* and very little in *c*. Some of the variation in *w* is tentatively explained by group behavior phenomena.

From the relationship between *w* and *cal*, it is determined that the minimum energy requirement of rat food is about 137 calories per 100 gm. food.

The relationship between *cal* and *c* is somewhat suggestive of a hyperbolic function. If this hypothesis is valid, then it follows that a rat would strive for a definite amount of food *value* per day, rather than food *volume*.

Combining the two relationships described above, it is determined that rats require about 18.9 grams of moderately high caloric food per 100 gm. body weight per day. Therefore, the average (250 gm.) rat would need about 47 grams of food daily, and this figure agrees reasonably well with other estimates of rat intake. Despite the fact that about half (author's estimate) of the 150 gm. daily ration falls below the minimum energy requirement of 137 calories per 100 gm. of food,

the quantity of food available was clearly in excess of rat needs. Therefore, it is suggested that food distribution, rather than quantity, may have governed the population of the block under study.

A close relationship was obtained between w and p. This is interpreted as meaning that rats prefer foods on which they will gain weight. It is suggested that some of the scatter in the points is due to avitaminosis, aversion to highly spiced foods, preference for sweetened foods, or a combination of these factors.

REFERENCES

ALLEE, W. C., EMERSON, A. E., PARK, O., PARK, T., AND SCHMIDT, K. P., 1949. *Principles of Animal Ecology*. Saunders & Company, Philadelphia; 837 pp.

BOWES, A. DEP., AND CHURCH, C. K., 1946. *Food Value of Portions Commonly Used*. A. DeP. Bowes, Philadelphia; 6th Ed.; 58 pp.

BRIDGES, M. A., AND MATTICE, M. R., 1950. *Food and Beverage Analysis*, Lea and Febiger, Philadelphia; 3rd Ed.; 412 pp.

CHITTY, D., 1942. A relative census method for brown rats (*Rattus norvegicus*), *Nature* **150**: 59–60.

EMLEN, J. T. JR., STOKES, A. W., AND DAVIS, DAVID E., 1949. Methods of estimating populations of brown rats in urban habitats, *Ecol.* **30**: 432–442.

MALTHUS, T. R., 1798. *An Essay on the Principle of Population*. Johnson, London (also 1909, in Economic Classics, Macmillan, New York).

ORGAIN, H., AND SCHEIN, M. W., 1953. A preliminary analysis of the physical environment of the Norway rat, *Ecol.* **34**: 467–473.

THE RELIABILITY OF "VISUAL SURVEYS" IN EVALUATING FLY DENSITIES FOR COMMUNITY CONTROL PROGRAMS

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Since its advent in 1947, the grill method (Scudder, 1947) has become widely adopted as the most acceptable means of appraising the effectiveness of community fly control programs. Even though this method has been found satisfactory, the excessive time and man power required have elicited objection to its use from both large communities (population 50,000 and above) and small towns (population 5,000 to 10,000). In metropolitan areas, the inability to survey the greater part of the city within a 7- to 10-day period was the underlying reason, whereas in small towns the objection concerned the time element together with a general dislike of the grill itself. In these small-community programs, the individual in charge of operations is selected primarily for his ability to supervise control activities and secondarily for his qualifications in the field of appraisal. Therefore, even though dislike of an acceptable evaluative tool is not valid justification for neglecting its usage, nevertheless, from a practical standpoint, it is of paramount importance. Moreover, field observations indicated that the "reconnaissance" or "visual survey" method might combine the merits of reasonable accuracy with greater speed. Comparative studies which were undertaken in three cities to determine the relative efficacy of the two techniques also showed (Schoof, unpubl.) that reconnaissance surveys based on "estimated grill counts" yielded data comparable to those derived from grill inspections.

This paper describes the more detailed experiment which was designed to provide conclusive information concerning: 1) the degree of correlation between "estimated" and actual grill counts over the same fly attractants; and 2) the similarity and differences in fly prevalence data obtained with the two methods by individuals and by inspection teams. The tests were conducted at the Communicable Disease Center's Fly Control Project at Phoenix, Arizona, in 1951.

METHODS

The experimental design consisted of simultaneous examination of 50 attractants per week by two inspectors, each using the two methods. Data were collected in an orderly fashion to minimize bias and to make the four observations per attractant as independent as possible. The two members of the inspection team first recorded an "estimated grill count," then each made an actual grill count of the flies present at each attractant. To obtain the "estimated grill count," the inspector made a visual observation of the fly concentration and then assigned to that concentration the count he felt would have been obtained if a Scudder

[1] The authors are particularly indebted to Dr. L. Otis Emik for assistance and advice on various aspects of this study.

grill had been placed over the attractant. The grill was then placed over the attractant site and the flies which alighted upon it were counted.

The surveys were conducted in a sample of blocks in which were represented those attractants which normally occur within city blocks, and of the fly density ranges which characterize the project city. One day's survey for each inspector consisted of observations on 50 fly attractants.

TABLE 1

Average number of flies per grill for two methods by teams of two inspectors, Phoenix, Arizona, 1951

PERIOD OF INSPECTION	TEAM	INSPECTOR	NUMBER ATTRACTANT SITES	AVERAGE NO. FLIES PER GRILL BY	
				"Visual estimate"	Actual count
July 25–Oct. 22	1	A	600	32.3	35.5
		B		25.6	33.1
May 25–July 9	2	A	400	14.0	15.3
		C		12.8	14.7
Sept. 9–Oct. 23	3	D	350	25.7	30.8
		E		25.1	30.7
July 23–Aug. 22	4	D	250	32.8	43.6
		F		32.9	36.5
June 8–July 17	5	D	350	19.3	26.8
		G		23.0	26.3
June 20–Aug. 16	6	H	350	33.0	34.7
		J		32.3	33.6
Sept. 7–Oct. 24	7	H	350	27.0	28.8
		K		27.0	27.8
May 24–June 12	8	H	200	4.1	4.5
		L		4.0	4.1

The data were collected by the inspectors routinely employed to conduct reconnaissance and grill surveys on the control program. None of the inspectors were professional entomologists or biologists, the majority having been hired and trained locally. The normal exigencies of an operating control program necessitated a number of personnel changes which modified team composition.

STATISTICAL METHODS

The statistical methods and symbols employed in this paper follow the basic principles of correlation and covariance as given by Snedecor (1946) and the 2 x 2 chi-square method for correlated observations as given by McNemar (1949).

Data were collected by 8 two-man teams from 2,850 fly attractant sites and were used for comparisons of actual grill counts to estimated grill counts, and of one inspector to the other. The samples varied from 200 to 600 attractant sites per team. The variation in fly prevalence is shown in Table 1.

Frequency distributions were made of fly counts, by methods, for the 8 teams to determine the transformation which would be appropriate for the methods

TABLE 2

Correlation statistics between actual (Y) and visual estimates (X) of fly grill counts using coded values

TEAM	INSPECTOR	SUMS, SUMS OF SQUARES AND PRODUCTS					DEGREES OF FREEDOM	CORRELATION COEFFICIENT
		SX	SY	Sx^2	Sy^2	Sxy		
1	A	3292	3309	1393.90	1633.86	1357.62	598	0.900
	B	3097	3258	1363.32	1643.06	1343.29	598	0.898
2	A	1343	1368	2291.88	2519.44	2259.94	398	0.941
	C	1379	1353	2010.90	2360.48	2044.53	398	0.939
3	D	1843	1873	860.29	991.77	812.32	348	0.880
	E	1818	1868	774.79	960.22	773.07	348	0.896
4	D	1392	1440	655.34	821.60	645.08	248	0.879
	F	1392	1419	639.34	710.76	601.01	248	0.891
5	D	1457	1490	1785.72	2116.86	1797.34	348	0.924
	G	1537	1510	1919.37	2143.43	1863.94	348	0.919
6	H	2002	1993	802.56	984.29	814.04	348	0.916
	J	1967	1987	816.46	916.52	771.06	348	0.892
7	H	1875	1887	736.36	783.37	660.07	348	0.869
	K	1861	1860	777.80	819.43	689.11	348	0.863
8	H	435	407	502.88	588.76	451.78	198	0.831
	L	420	402	490.00	547.98	456.80	198	0.881

employed in the statistical analyses. The logarithmic transformation was found to be satisfactory for analyses of these biological data. To facilitate the many computations, the counts (0–1200) were coded into 13 class values using intervals of a logarithmic scale. The distribution of these transformed data was approximately "normal."

RESULTS

Relationship between methods. Unpublished data from previous studies showed the relationship between the visual and the grill method of surveillance to be rectilinear. The coefficient of correlation (a measure of the degree of association of one method with the other method) was determined for each inspector. The results are shown in Table 2. Highly significant correlations between methods were

found for all teams. Inspector by inspector, the visual method agreed with the actual grill counts with a range of 69 to 89 per cent accuracy. Team 2 showed the highest relationships, whereas team 8 showed the lowest. The coefficients of correlation between methods by inspectors within each team were not significantly different in any of the 8 teams by Fisher's z test.

Relationship between inspectors. The comparisons of inspectors by methods are shown in Table 3. The relationships between inspectors were highly significant for all teams by both methods; which is to say, the two inspectors in each team were in close agreement in determining the number of flies both by actual grill count and by visual estimate. The range of accuracy was 89 to 98 per cent for actual grill counts and 75 to 91 per cent for visual estimates. The inspectors in team 2 again were in closer agreement in determining the number of flies than were those in any other team in the study.

TABLE 3

Correlation statistics between inspectors for fly grill counts by two methods using coded values

Team	Inspector		Degrees of freedom	Correlation coefficient	
	(X)	(Y)		"Visual estimate"	Actual
1	A	B	598	0.889	0.960
2	A	C	398	0.953	0.989
3	D	E	348	0.893	0.967
4	D	F	248	0.873	0.943
5	D	G	348	0.933	0.983
6	H	J	348	0.878	0.962
7	H	K	348	0.867	0.948
8	H	L	198	0.889	0.975

Simulated control threshold. In most field programs, control measures are instituted when the grill count reaches or exceeds a specific number of flies. This "threshold value" varies by location and season according to the judgment of the entomologist.

Further tests of the comparability of the visual method and the grill method were made by applying theoretical threshold values of field practice to the survey data. Using threshold values of 11, 6, or 3 flies per attractant, no significant differences between methods were found. The inspectors were in agreement in indicating the necessity for control action, with the exception of one team. The inspectors in team 1 differed significantly by the chi-square test for the visual method. Although these inspectors detected changes in numbers of flies with equal accuracy one consistently counted more flies than the other. Hence, two different values must be used by these inspectors, for the administration of consistent control measures.

DISCUSSION

The previous findings establish that the reconnaissance or visual survey method can provide data which are of parallel accuracy and reliability to those

yielded by the grill survey technique. This correlation enables the reconnaissance survey to accomplish the objectives of the grill survey method, namely, to guide and to measure the relative effectiveness of the control operations. Whether untrained individuals could utilize the visual method nearly as accurately with the same number of observations as has been shown with experienced inspectors is yet to be determined.

The principal advantage of the reconnaissance technique over grill coverage is that it enables the inspector to cover a greater amount of area within the same time. Under average circumstances, grill surveys require 15 to 20 minutes per block, whereas a visual inspection of a block can be completed within 5 to 7 minutes. The greatest economy in time is effected in blocks with alleys. The time saved by the reconnaissance system can be utilized to permit more complete coverage of the municipality or it can be converted to other phases of the control program, such as sanitation or chemical control.

Aside from the above advantage, the principal benefit from visual coverage lies in its greater acceptability to the small-community program. Towns which tend to neglect entomologic appraisal are more prone to accept the visual survey than one employing grill inspections.

The chief drawback to visual inspections is the placement of a greater premium upon the ability of the individual inspector. The fact that the grill provides a visible yardstick is a safeguard which retards digression toward haphazard and erroneous inspections.

To derive the benefits of both techniques, community fly appraisal should include elements of each method. Basically, each inspector would be trained in the use of the grill, then instructed in the visual method of inspection. Following this, routine surveillance of the community could be accomplished by the reconnaissance technique, the latter being subject to periodic standardization with the grill technique in much the same manner as quantitative equipment is calibrated against fixed standards. In large cities where several inspectors are employed, further standardization among individuals might be required so as to establish uniform fly count relationships with the designated control threshold.

SUMMARY

The relations were determined between the number of flies "visually estimated on a grill count basis" and then actually counted with a Scudder grill on 2,850 fly attractant sites. Eight two-man teams of inspectors collected these data throughout the 1951 "fly" season in Phoenix, Arizona.

The visual estimate technique agreed with the grill method by the same inspector with a range of 69 to 89 per cent accuracy. Agreement between inspectors was 89 to 98 per cent using actual counts, and 75 to 91 per cent using visual estimates. By application of simulated field threshold values to indicate the necessity for control action, the inspectors, with the exception of one team for visual method, were in agreement.

The results of this study show that the "visual estimate" technique is a useful extension of the Scudder grill method for appraising adult fly densities.

REFERENCES

McNemar, Quinn, 1949, *Psychological Statistics*, John Wiley and Sons, Inc.

Schoof, Herbert F., Survey and appraisal methods for community fly control programs (Unpublished).

Scudder, H. I., 1947, A new technique for sampling the density of house fly populations, *Pub. Health Rep.* **62:** 681–686.

Snedecor, G. W., 1946, *Statistical Methods*. 4th ed. Iowa State College Press, Ames, Iowa.

A RAPID PERMANENT-MOUNT STAIN TECHNIC FOR THE DIAGNOSIS OF THE INTESTINAL PROTOZOA[1]

A Preliminary Report

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There has been a long-felt need for a staining technic for intestinal Protozoa that would be comparable in effectiveness and in speed of preparation with simple-smear methods now in common use, that would be simple and dependable enough for employment by technicians not especially trained in its use, that would demonstrate good diagnostic details in both trophozoites and cysts, and that would provide permanent preparations for file purposes. Simple-smear methods have the disadvantage of being temporary, and the iron hematoxylin methods in current use are time-consuming, require skill in their use and may give variable results on different stools and with different batches of stain.

A two-and-a-half minute permanent staining technic is herein described which nearly approximates the fineness of detail seen in iron hematoxylin preparations. The staining solution is simple to prepare, few ingredients are required and technical skill is not necessary. No ripening process is required and the preservation and fixation properties are combined with the dyes. The appearance of the stained preparation is similar to that obtained with the iron hematoxylin methods. The dyes selected for use in the staining solution produce a bluish-purple fecal background. Parasites stain a variable blue to purple in color.

The following ingredients are required to make 1,000 cc. of the fixing and staining solution:

Acetone	50 cc.
Glacial acetic acid	50 cc.
Formaldehyde solution USP	10 cc.
Schaudinn's solution[2]	890 cc.

To this solution add 1.25 gram of acid fuchsin and .5 gram of fast green FCF, mix, and the solution is ready for use. It should be stored in a tightly stoppered bottle, preferably a brown one. After two months' preparation the solution still appears stable and the results obtained with it are unchanged. Uniformly good results have been obtained on all types of stools thus far tested, regardless of consistency or pH.

Technic. The method described below has been found useful for routine diagnosis of the intestinal Protozoa. At the completion of the slide examination the

[1] The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

[2] Schaudinn's solution: saturated solution bichloride of mercury, 2 parts; 95% alcohol, 1 part.

stained preparations have usually been discarded; however, several specimens have been filed for permanency evaluation. Thus far, slide preparations have been observed over a period of two months with no apparent change in diagnostic or staining characteristics.

(1) Using an applicator stick, smear a thin layer of feces on a glass microslide as recommended for permanent-mount preparations. (At no time during the staining procedure should the fecal smear be allowed to dry.)

(2) Using a medicine dropper or a glass-stoppered dropper bottle, immediately cover the fecal preparation with the staining and fixing solution, being sure that the preparation is completely covered.

(3) Gently heat the stain-covered smear over an alcohol lamp or laboratory burner until steam is first observed. (Two or three slow passes of the fecal smear through a flame is usually sufficient. Do not boil and do not allow staining solution to flame.)

(4) Immediately wash gently under tap water.

(5) Place in 50% and 70% alcohol for 30 seconds each, 95% and absolute alcohol for 15 seconds each, and clear in xylol for one minute.

(6) Mount in Canada balsam. The preparation is now ready for examination.

Staining reaction. The staining characteristics of the intestinal Protozoa are similar to those of iron hematoxylin preparations. Nuclear detail and chromatoid substance are particularly clear. Cysts of all species stain well, and also the trophozoites thus far seen. The trophozoites of the amebae, except *Dientamoeba fragilis*, have been observed, as well as those of *Giardia lamblia*. Evaluation of this staining technic by comparison with the MIF (Sapero and Lawless, 1953), in a brief parallel study, has given promising results.

REFERENCES

SAPERO, J. J., AND LAWLESS, D. K., 1953. The "MIF" stain-preservation technic for the identification of the intestinal Protozoa, *Am. J. Trop. Med. and Hyg.* **2**: 613–619.

CORRESPONDENCE

October 21, 1953

The Editor
The American Journal of Tropical Medicine and Hygiene

Dear Sir:

The paper by Myatt, Hernandez and Coatney on Studies in Human Malaria, No. XXXIII, in the September issue of the Journal has three mistakes due to a printer's error. In Tables 1, 2, and 3, on pages 789 and 790, the bar-box showing Daraprim 25 mg. daily extends through only 5 weeks of the test; it should have extended through 7 weeks as indicated in the legend.

Yours truly,

G. Robert Coatney
Laboratory of Tropical Diseases
National Microbiological Institute

BOOKS RECEIVED

Note: Books received for editorial consideration will be intermittently listed. This acknowledgment must be regarded as an adequate expression of appreciation for the courtesy of the author or publisher. Selections will be made for review in the interest of our readers.

First International Symposium on Yaws Control, Bangkok, 1952, WHO Monograph Series No. 15. 418 pp., illustrated. Geneva. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, New York, 1953. Mixed language edition. Price $4.50, Fr. fr. 1,440., Sw. fr. 18.

Die Unspezifischen Bluteiweissreaktionen. Kolloid-habilitätsreaktionen im Serum und Plasma als Hilfsmittel zur Krankheitserkennung, by Priv. Doz. Dr. Med. Fritz Heepe. Vol. 61, Wissenschaftliche Forschungsberichte. 241 pp. with a bibliography of some 4,500 references. Darmstadt, Verlag Dr. Dietrich Steinkopff, 1953. Price DM 30.-

Insecticides. Manual of Specifications for Insecticides and for Spraying and Dusting Apparatus. Loose leaf binding, to be supplemented through Dec. 31, 1955. Geneva, WHO. Distributed by International Documents Service, Columbia University Press, 2960 Broadway, New York 27, New York. Price $8.50, £3, Fr. fr. 2800, Sw. fr. 35.

Clinical Tropical Diseases, by A. R. D. Adams and B. G. Maegraith, from the School of Tropical Medicine, Liverpool. 508 pp., illustrated. Springfield, Ill., Charles C. Thomas, 1953. Price $10.50.

The Housefly, by Luther S. West. 584 pp. illustrated. Ithaca, New York. Comstock Publishing Co., 1951. Price $7.50.

A Guide to Human Parasitology for Medical Practitioners, by D. B. Blacklock, C.M.G., M.D. (Edin.); D.P.H. (London); D.T.M. (Liver.) and T. Southwell, D.Sc., Ph.D., A.R.C.Sc., F.Z.S., F.R.S.E.; revised by T. H. Davey, O.B.E., M.D. (Belf.); D.T.M.

(Liver.). 5th edition, 228 pp. illustrated with 3 plates in color and 120 text illustrations. Baltimore, Williams & Wilkins Co., 1953. Price $5.50.

Bacterial Genetics, by Werner Braun, Ph.D. 238 pp. illustrated with 67 fig. Philadelphia and London, W. B. Saunders Co. 1953. Price $6.50.

Nursing, A survey of recent legislation. Reprint from International Digest of Health Legislation, 1953, 4: 461–498. 34 pp. Geneva, WHO, 1953. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, New York. Price $.25, Sw. Fr. 1.-; 2/-

The Conquest of Plague, a study of the evolution of epidemiology, by L. Fabian Hirst, M.D. Lond., with a foreword by Lieut.-Gen. Sir William Macarthur. 478 pp., New York: Oxford University Press, 114 Fifth Avenue, 1953. Price $11.00.

BOOK REVIEWS

Survey of Antimalarial Agents. Chemotherapy of *Plasmodium gallinaceum* infections; toxicity; correlation of structure and action. Public Health Monograph No. 9. By G. Robert Coatney, W. Clark Cooper, Nathan B. Eddy and Joseph Greenberg. 323 pp. Washington, D. C., Federal Security Agency, Public Health Service, P.H.S. Publication 193, Superintendent of Documents. Price $1.25.

Since 1941, nearly 4,000 substances have been screened for antimalarial activity against *Plasmodium gallinaceum*. Some have been studied clinically and reported in a long series of papers in various journals. The main purpose of these studies was to "arrange the compounds as consistently as possible according to chemical relationships and to analyze the accumulated data for the light that may be thrown on the relation between antimalarial activity and chemical structure." The tabulated data present the degree of toleration of the drugs by chicks, their antimalarial activity and the therapeutic index of each, that is, the maximum tolerated dose divided by the minimum effective therapeutic dose.

This imposing piece of work deals with fourteen groups of compounds arranged in 129 tables. A general summary reviews all of the compounds which showed significant antimalarial action. Seventy-seven compounds had a therapeutic dose approximately equivalent to that of quinine, three-quarters of which were amino-quinolines. However, the order of effectiveness and of toxicity was very different. Seventy had a therapeutic index greater than that of quinine, only 32 of which were amino-quinolines, 34 being amino alcohols. The compound with the largest therapeutic index was a sulfanilamidopyrazine. Fifteen compounds showed true prophylactic action against sporozoite-induced infections. A group of benzene thiocarbamides were partially prophylactic and yet had no therapeutic action.

This useful investigation adds much information to the important Wiselogle survey of antimalarial drugs published in 1946.

L. W. Hackett

Human Factors in Air Transportation. Occupational Health and Safety, by Ross A. McFarland, Ph.D. Associate Professor of Industrial Hygiene, Harvard School of Public Health. pp. xv + 830, illustrated. New York, Toronto, London: McGraw-Hill, 1953. $13.00.

McFarland has covered the entire field of health as related to the air transportation industry, and a good many other things as well. Since a very wide range of activities is included, many other industries can draw much useful information from this work, particularly the portions on psychological tests and medical examination in personnel placement; the use of periodic medical examinations; accident prevention in shops and other ground installations; sanitation; and the organization of industrial health programs.

The book is addressed to both medical and non-medical people who have responsibilities

in management. It is more inclusive than critical. The physician new to the specialty of industrial medicine, or its subdivision, aviation medicine, can rely on this book as his principal reference. The private physician looking for advice concerning a patient who plans a trip by airplane or who contemplates working in aviation will find most of his questions answered here. The industry executive can get a clear indication of what to expect from (and what not to ask of) a medical program, and indications of the associated costs.

There is but one serious omission; it is one which is more of feeling than of fact, and perhaps debatable. It arises from the effort to be objective, and is manifest in many statistical tables and similar ways. At many points, one has the sense of discussing biological entities en masse, rather than people. Such an attitude is certainly not uncommon, but it is unfortunate in a book with such potentialities of wide influence as this one. In the discussion of periodic medical examinations, much emphasis is placed on the discovery of deviations from normal or baseline values of weight, blood count, etc., but little is said of the importance of offering an opportunity for discussing personal problems of various kinds. Whereas pages are devoted to certain technics of physical and laboratory examination, this important matter is treated only by a short quotation from Karl Menninger. The constructive mental health value of permitting people to talk out their anxieties and antagonisms is not brought out as it should be.

The author has not been afraid to repeat himself where this is necessary in bringing related materials into proper context under the various headings. The index is good. The high standard of bookmaking which we have learned to expect of McGraw-Hill has been maintained. Altogether, this is a worthy companion for McFarland's earlier *Human Factors in Air Transport Design*, and makes the air transport industry the best equipped of any with respect to access to pertinent knowledge of human biology.

RODNEY R. BEARD

Amebiasis. Pathology, Diagnosis, and Chemotherapy, by HAMILTON H. ANDERSON, M.D., WARREN L. BOSTICK, M.D., and HERBERT G. JOHNSTONE, Ph.D., University of California Medical Center, San Francisco, California. Published by Chas. C. Thomas, Springfield, Illinois, 1953. Price $11.50.

This monograph encompasses a discussion of amebiasis not only from the standpoints of pathology, diagnosis and chemotherapy, as stated in the title, but from the aspects of epidemiology and pathogenesis as well. The material is, in general, brought up to date, but with a striking unevenness. For example, of the 431 pages, only 23 are devoted to symptomatology and clinical manifestations. Fifty four pages are justifiably given to laboratory diagnosis, but little space is given to the important problem of basic diagnosis of the patient. The recognition of *E. histolytica* is of paramount importance in diagnosis but so is the establishment of the symptomatology on the basis of this finding. The development of the problem of differential diagnosis receives little attention, although there is a half-page discussion of the association of amebiasis and colonic malignancy. On page 235 there is a description of the gross differentiating factors in the stools of bacillary and amebic dysentery. These differential points are present in certain instances but the reader is led to believe that the features given will be more or less generally present and helpful. This is not so. Likewise the discussion of sigmoidoscopy on page 236 describes the procedure as a useful adjunct, and the implication is given that the instrument should be employed where amebas have not been found on stool examinations. Whether amebas are present or not the procedure is important, for it is useful in ruling in or out diseases other than amebiasis. The finding of *E. histolytica* may establish a diagnosis but not necessarily the diagnosis responsible for the complaints. The criticisms which have been given on diagnosis, differential diagnosis, and symptomatology suggest that these sections of the monograph were not gone over nor developed adequately by a clinician, a process which would probably have improved them materially.

There are many instances of carelessness in editing. For example, on page 284 Goldman is twice referred to as Golden; the term pathology is often used to mean pathological findings.

In general, the authors have brought together the newer knowledge of amebiasis with sufficient clarity to make this text a suitable reference volume. The current literature until 1951 has been well combed in a very helpful bibliography.

W. A. SODEMAN

Modern Concepts of Leprosy, by HARRY L. ARNOLD, JR., M.D., F.A.C.P., Department of Dermatology, Straub Clinic, Honolulu, Hawaii. Publication No. 145, American Lecture Series. 105 pp. with 33 illustrations. Springfield, Illinois: Charles C. Thomas. 1953. Price $3.75. Published simultaneously by Blackwell Scientific Publications, Ltd., Oxford, England and The Ryerson Press, Toronto, Canada.

This is an excellent 91-page, well illustrated monograph on leprosy, in which the various phases of the disease are well and concisely discussed. It should be made available to medical students, physicians and public health personnel. Such individuals must become leprosy conscious since the United States has members of the armed services stationed in many areas in the world in which the disease is endemic. Based on past experience, it is quite likely that some of them will become infected and develop the disease clinically, no matter in what section of the country they may reside.

Due to the protean manifestations, leprosy may simulate many of the more common dermatological and neurological diseases. In the chapter on diagnosis the author has given valuable assistance in differential diagnosis. Early diagnosis is essential in order that treatment may be instituted early in the course of the disease.

Control measures should be based on available factual knowledge of the factors influencing transmission. The present information relative to those factors is well summarized in the author's statement that "the epidemiology of leprosy permits us to make very few positive statements about it."

L. F. BADGER

Rattenbiologie und Rattenbekämpfung einschliesslich der Toxikologie gebräuchlicher Rattengifte, by STEINIGER, Reg. Rat. Dr. habil. FRITZ. 149 pp. 33 illustrations. Stuttgart, Germany. Ferdinand Enke Verlag. 1952. Price DM 11.

Dr. Steiniger discusses the biology, control, organization for control, and disease vectored by the "wanderratte" (Norway rat) and the "hausratte" (roof rat). He briefly mentions a large species of vole or meadowmouse and the American muskrat which was introduced into Europe some score years ago. He has skillfully condensed considerable information in his 149 pages. In his chapter on organization for rat control he stresses poisoning programs. The section of baits, poisons and traps occupies 73 pages. He discusses the importance of rat-proof construction and harborage removal in 10 pages and 11 figures after remarking that this phase of rat control has had too little attention in Germany to date. His account of means of rat proofing sewer and drainage systems presents several helpful new figures.

In a comparison of general public campaigns of rat control and the role of limited individual control, he gives advantages and disadvantages of each. The individual attempts generally fail. The usefulness is mentioned of experienced professional pest control organizations as a year long deterrent to large rat populations.

CARL O. MOHR

First Report of the Expert Committee on Influenza. World Health Organization Technical Report Series No. 64. Available also in a French Edition. 32 pp. Geneva: W. H. O., Palais des Nations, or Columbia University Press, International Documents Services, 2960 Broadway, New York 27, N. Y. 1/6, $0.20; Sw. fr. 0.80; Fr. fr. 65.

"Influenza is one of the most important diseases still unconquered." Recommendations concerning methods of control and prevention are contained in this report. The report indicates that the best approach to the prevention of influenza lies in prophylactic immunization and that little hope may be expected from quarantine procedures. The methods for preparing the vaccine are still in the developmental stage and further research will be needed

to select the proper strains for inclusion in the vaccine, to develop appropriate production and quantitative potency-assay procedures, and to determine the best method of administering the material to man. Hope is expressed for the usefulness of adjuvants in broadening the antigenic response and in extending the duration of immunity. In discussing influenza therapy, the role of antibiotics receives particular attention in the report.

The specialized laboratory-worker will find useful the detailed descriptions of the complement-fixation and hemagglutination tests for the diagnosis of influenza, and the methods of preparation of antisera for the comparison and typing of strains which are included in annexes to the report. The clinician and health worker will be interested in the new views on notification and speculations regarding the possibility of controlling severe pandemics of influenza.

W. H. O. Publication Notice

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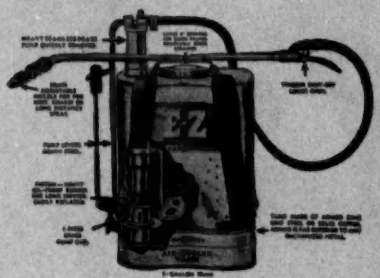

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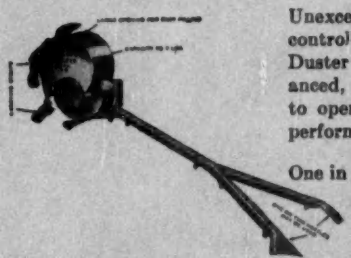

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